Prognostic Impact of the CD34+/CD38- Cell Burden in Patients with Acute Myeloid Leukemia receiving Allogeneic Stem Cell Transplantation

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Introduction: In acute myeloid leukemia (AML), leukemia initiating cells exist within the CD34+/CD38- cell compartment. They are assumed to be more resistant to chemotherapy, enriched in minimal residual disease cell populations, and responsible for relapse.

Purpose: We evaluated clinical and biological associations and the prognostic impact of a high diagnostic CD34+/CD38- cell burden in AML patients receiving an allogeneic stem cell transplantation (HSCT) in complete remission. Here, the therapeutic approach is mainly based on immunological graft-versus-leukemia effects.

Methods: Percentage of bone marrow CD34+/CD38- cell burden in 169 AML patients at diagnosis was measured using flow cytometry. The optimal cutoff of 6% was applied and used to evaluate the impact of a high CD34+/CD38- cell burden on outcome.

Results: The CD34+/CD38- cell burden and was highly variable (median 0.5%, range 0-89% of all mononuclear cells). A high CD34+/CD38- cell burden at diagnosis associated with worse genetic risk and secondary AML. Patients with a high CD34+/CD38- cell burden had shorter relapse-free and overall survival, which may be mediated by residual leukemia initiating cells in the CD34+/CD38- cell population, escaping the graft-versus-leukemia effect after allogeneic HSCT.

Conclusion: Evaluating the CD34+/CD38- cell burden at diagnosis may help to identify patients at high risk of relapse after allogeneic HSCT. Further studies to understand leukemia initiating cell biology and develop targeting therapies to improve outcomes of AML patients are needed.

Einleitung / Introduction

Epidemiology and AML diagnosis

Acute myeloid leukemia (AML) is a disease originating from myeloid progenitor stem cells that undergo uncontrolled proliferation and have lost their ability to differentiate. Although AML is a rare disease with an incidence of 3.8 cases per 100,000 individuals, it belongs to the most frequently diagnosed hematologic disorders[1,2] and often affects older individuals with a median patient age of around 70 years at diagnosis.[1-3] Without therapy, all AML patients die within a year after diagnosis,[4] and it was not until the 1960ies, with the introduction of chemotherapeutic agents such as cytarabine or daunorubicine, when remissions were achieved for the first time.[5,6]

AML patients present with a variety of nonspecific symptoms including fatigue, bleeding or infections, that usually are a result of peripheral cytopenia. With the transition of leukemic blasts from bone marrow into the peripheral blood, an elevation of the white blood count (WBC) can occur. This may lead to leukostasis if the WBC exceeds 100,000 Gpt/l and subsequently cause vascular occlusions. Leukemic cells can also infiltrate extramedullary tissue such as the skin or central nervous system (chloroma).[1] Besides *de novo* origins, AML often develops following other hematologic disorders – especially myelodysplastic syndromes (MDS) and myeloproliferative neoplasia (MPN) – and also occur frequently after cytotoxic therapy for unrelated neoplasms. This is particularly the case after the use of alkylating agents (with a peak 5-10 years after exposure) or topoisomerase-II inhibitors (with a peak 2-3 years after exposure).[1]

Morphologic identification, as well as immunohistochemical analyses of blasts in bone marrow and peripheral blood allow a discrimination between AML and acute lymphoblastic leukemia or MDS. Regarding to the World Health Organization (WHO) classification, the diagnosis AML requires the myeloid bone marrow blast count to exceed 20%.[7] An exception represent the AML-defining aberrations: in the cytogenetic subgroups t(8;21)(q22;q22) and

inv(16)(p13q22) or its variant t(16;16)(p13;q22), in which AML is diagnosed independently of the blast count. Besides the sole quantification of blasts in bone marrow and peripheral blood, morphologic analysis can divide patients into different French-American-British (FAB) types based on the degree of blast maturity and/or differentiation.[8] Since the FAB classification does not provide prognostic information, today, it is mainly used out of historic reasons.

Therapeutic options in AML

Curative AML treatment approaches are based on cytotoxic therapy and divided into one or more induction cycles.[1] The purpose of an induction therapy is the achievement of a complete remission (CR), that is characterized by normalization of bone marrow blast count (<5%) and the recovery of cell counts in peripheral blood.[1] Afterwards, consolidation therapies are used to maintain CR. Chemotherapeutic substances of choice are cytarabine combined with an antracycline (e.g. idarubicine, daunorubicine or mitoxanthrone).[9] Besides chemotherapy, possible consolidation therapies also include autologous or allogeneic HSCT.[1]

If an autologous HSCT is planned, patients in CR receive granulocyte-colony stimulating factor – with or without a prior cycle of chemotherapy - to mobilize CD34+ hematopoietic stem cells (HSCs) into the peripheral blood. During an apheresis, these CD34+ cells are collected and frozen. Afterwards, a myeloablative course of chemotherapy is applied and the following reinfusion of autologous HSCs secures hematologic regeneration.[1] During an allogeneic HSCT, HSCs from a sibling or unrelated donor provide this stem cell support.[1,10] Donor selection is based on a matching human leukocyte antigen (HLA) type. Over the years, HLA typing evolved with the recognition of new alleles and techniques changing from serologic assays for protein detection to molecular methods defining HLA genes by their deoxyribonucleic acid (DNA) sequence. Today, high resolution typing of the class I antigens

HLA-A, HLA-B and HLA-C and the two class II antigens HLA-DRB1 and HLA-DQB1 are referred to as gold standard.[11] In contrast to autograft procedures, the therapeutic effect of an allogeneic HSCT is believed to - in addition to the conditioning chemotherapy - also rely on graft-versus-leukemia (GvL) effects, in which donor lymphocytes recognize disparate minor histocompatibility antigens and eliminate surviving tumor cells.[12] The GvL effect may maintain CR but also harbors the risk of graft versus host disease (GvHD), a condition where donor's lymphocytes react against the patient's healthy tissue.[10,13]

The intensity of distinct conditioning regimens prior to allogeneic HSCT vary substantially and till today, no full consensus about a nomenclature was found. The Center for International Blood and Marrow Transplant Research (CIBMTR) classified conditioning regimens as myeloablative (MAC), reduced-intensity (RIC) or non-myeloablative (NMA) with a fluent passage between each other.[14] In MAC-HSCT, the administered dosage of chemotherapy and/or total body irradiation (TBI) eradicates the host hematopoiesis. Due to the high toxicity in this procedure only younger patients without relevant comorbidities are eligible.[15] With the introduction of NMA conditioning regimens, allogeneic HSCT became available for older patients or individuals with relevant comorbidities.[16,17] In contrast to MAC procedures, considerable less peripheral granulocytopenia is observed and autologous regeneration remains possible also without stem cell support. Here, the administered chemotherapies and/or TBI have little antileukemic activity[17] and the therapeutic success of this procedure nearly exclusively relies on GvL effects. As a result of a still existing host bone marrow, graft rejection more often occurs after NMA-HSCT and higher dosages and combinations of immunosuppressive drugs are needed.[17] Finally, the dose intensity of RIC regimens lies between MAC and NMA conditionings. Still, the chronology in all HSCT regimens is rather similar with peripheral count regenerations approximately 10-14 days after HSC infusion.

Table 1 provides a summary of definitions and examples for each regimen.

Table 1: Comparison between MAC, RIC and NMA conditioning regimens, modified after Giralt *et al.*[14] and Gyurkocza *et al.*[18]

	Myeloablative Conditioning (MAC)	**Reduced-Intensity Conditioning (RIC)**	**Non-myeloablative Conditioning (NMA)**
Description	involves a myeloablative course of chemotherapy no autologous recovery possible consists of alkylating agents with or without TBI	does not fit MAC or NMA conditioning characteristics: causes potentially prolonged cytopenia may require stem cell support dose of alkylating agents and/or TBI is reduced by ≥30% compared to MAC	causes only minimal cytopenia does not require stem cell support
Examples* [16,19,20]	12 Gy TBI + Cyclophosphamide 2x60 mg/kg	Busulfan 8 mg/kg + Fludarabine 5x30 mg/m^2	2 Gy TBI + Fludarabine 3x30 mg/m^2
Required Immunosuppression			
Intensity/ Toxicity			

Abbreviations: Gy, Gray; kg, body weight (kg); m^2, body surface area (m^2); TBI, total body irradiation

* The chosen conditioning examples are the ones frequently used in patients at the University of Leipzig.

Within our publication, we included patients treated with MAC- (12 Gy TBI and Cyclophosphamide) and NMA-HSCT (2 Gy TBI and Fludarabine).

Genetic risk classification for therapeutic decisions in AML

AML displays a disease with a large genetic heterogeneity and enormously various clinical disease courses. Over the past years, the prognostic relevance of chromosomal aberrations and molecular mutations became clearer,[21] adding to the long known list of clinical prognostic factors such as age, performance status, comorbidities, primary *vs.* secondary disease or WBC and lactate dehydrogenase levels at diagnosis.[1,3,22] Today, cytogenetic analyses and prevalence of specific mutations are considered within the WHO classification, the most commonly used classification for AML (**Table 2**).[7]

Table 2: Adapted from Arber *et al*:[7] The 2016 revision of the WHO classification of AML

Acute myeloid leukemia and related neoplasms
Acute myeloid leukemia with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
AML with t(6;9)(p23;q34.1); *DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBM15-MKL1*
Provisional entity: AML with BCR-ABL1
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
Provisional entity: AML with mutated RUNX1
Acute myeloid leukemia with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Acute myeloid leukemia, not otherwise specified
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis
Myeloid leukemia associated with Down syndrome

Today, genetic risk classifications already influence treatment decisions in the clinical practice. There is an agreement that individuals with adverse risk AML should receive an allogeneic HSCT if a suitable donor is available. Due to the high cure rates of favorable risk AML with chemotherapy and higher treatment-related mortality in allogeneic HSCT, this procedure is usually not recommended for patients with favorable risk AML in first CR.[23] The optimal choice of consolidation treatment for intermediate risk AML remains controversial and is being further investigated in clinical trials. For example, a prospective randomized Phase III trial currently compares NMA-HSCT to chemotherapy in first CR for elderly patients (EudraCT Number 2007-003514-34). Besides the choice between chemotherapy and autologous or allogeneic HSCT in first CR, treatment options during relapses or later CRs might also include targeted therapies (e.g. novel kinase inhibitors or hypomethylating agents) and enrollment in studies testing experimental drugs with their various consequences regarding mortality, morbidity, quality of life, and financial costs. Therefore, the choice of the optimal treatment for each individual at diagnosis and during remission or relapse remains an everyday challenge for physicians.

In recent years, various risk stratification systems based on chromosomal aberrations – which are among the strongest risk factors - have been developed within the different cooperative groups (e.g. of the Southwest Oncology Group/Eastern Cooperative Group [SWOG/ECOG],[23] Medical Research Council [MRC][24] and Cancer and Leukemia Group B [CALGB]).[25] Although some varieties between the used classifications exist, several general statements can be derived.

About 25% of younger and 7% of older AML patients present with cytogenetics known to associate with relatively good outcomes.[26] All cooperative groups agree that the core-binding factor AMLs t(8;21)(q22;q22) and inv(16)(p13q22) or t(16;16)(p13;q22), as well as acute promyelocytic leukemia (APL) defined by the t(15;17)(q22;q21) translocation have a favorable prognosis.[1,25,27-31] These karyotypes appear more frequently in younger patients

and, depending on performance status and comorbidities, can exceed CR rates of 90% and long-term remission rates of 60% in younger and 30% in older individuals.[1,24-26]

On the other hand, 10-20% of younger and 20-30% of older patients have adverse cytogenetic abnormalities, including complex karyotypes (depending on the used classification system ≥3 or ≥ 5 unrelated aberrations in one clone), monosomies of chromosome 5 or 7 and aberrations on chromosome 3q.[1,25,26,28] Besides their higher incidence in older patients, they also appear more frequently in secondary or treatment-related AML.[1] For patients with these aberrations, outcome is poor with long-term remission rates of 10% for younger and 0-5% for older individuals.[24-26,32] Most of the remaining cytogenetic aberrations not fitting into the "favorable" or "adverse" risk groups were either not further classified (SWOG/ECOG, CALGB) or defined as standard or intermediate risk (MRC).

Approximately 40-50% of all patients show no aberrant cytogenetics but present with a normal 46,XX or 46,XY karyotype (CN). CN-AML was also defined as intermediate risk by all three mentioned cooperative groups.[23-25] Interestingly, subgroups with distinct long-term survival rates ranging from about 20-45% were found CN-AML patients.[27,28] Further analyses revealed that this striking heterogeneity can at least in part be explained by several molecular markers including gene mutations and aberrant gene expressions.[33]

Eventually, following the WHO 2008 recommendations,[34] three molecular markers were included into a novel risk classification system provided by the European LeukemiaNet (ELN) in 2010 to further differentiate the prognosis of CN-AML.[35] The most frequent gene mutations in AML are mutations in the nucleophosmin-1 (*NPM1*) gene (50-60% of CN-AML cases) which have a positive impact on survival.[7,36,37] The second mutation with favorable impact included into the ELN 2010 classification affects the CCAAT/enhancer-binding protein α (*CEBPA*) gene where N-terminal nonsense and C-terminal frame shift mutations can be distinguished.[38] *CEBPA* mutations appear in approximately 15% of CN-AML.[34] Internal tandem duplications (ITD) of the FMS-related tyrosine kinase 3 (*FLT3*) gene with variable

lengths which occur in 30-40% of CN-AML cases are believed to provide a proliferation and survival advantage for AML blasts.[34,39] In 2010, the ELN included these three molecular markers into risk stratification and proposed the following system (**Table 3**).

Table 3 (Adapted from Döhner *et al.*)[35]: Cytogenetic and molecular genetic risk classification in AML according to the ELN 2010 classification

Genetic group	Subsets
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD (normal karyotype) Mutated *CEBPA* (normal karyotype)
Intermediate-I	Mutated *NPM1* and *FLT3*-ITD (normal karyotype) Wild-type *NPM1* and *FLT3*-ITD (normal karyotype) Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL* Cytogenetic abnormalities not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1* t(6;9)(p23;q34); *DEK-NUP214* t(v;11)(v;q23); *MLL* rearranged –5 or del(5q) –7 abnl(17p) Complex karyotype*

* Three or more chromosome abnormalities in the absence of one of the WHO designated recurring translocations or inversions, that is, t(15;17), t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), inv(3) or t(3;3); indicate how many complex karyotype cases have involvement of chromosome arms 5q, 7q, and 17p

While the before mentioned core-binding factor leukemias and APL still were proposed as favorable, and deletion 5 or 7, abnormal chromosome 3 or 17p and complex karyotypes as adverse risk, CN-AML was further stratified by the molecular markers. CN-AML with a *CEBPA* mutation as well as CN-AML with presence of a *NPM1* mutation in the absence of a

FLT3-ITD was also defined as favorable risk. All other CN-AML, namely *FLT3*-ITD with or without *NPM1* mutations as well as *NPM1* wild type without *FLT3*-ITD were assigned to the intermediate-I genetic group. t(9;11)(p22;q23) and all other genetic abnormalities not defined as favorable or adverse formed the intermediate-II group. The prognostic significance of the ELN 2010 risk classification later was shown by several authors for both younger and older AML patients.[26,40-42] Given the observed inferior outcomes in patients over the age of 60 years and the distinct treatment intensities in chemotherapy protocols over or under 60 years of age, it was recommended to apply this classification for older and younger individuals separately.[26,40]

Immunophenotyping

Immunophenotyping constitutes another important modality to elucidate AML phenotypes. With the help of this biophysical laser-based technology, cells can be distinguished not only by size and granulation, but with the use of fluorescence-labeled antibodies the expression of distinct surface proteins can be detected. The clusters of differentiation (CD) mostly are membrane-attached glycoproteins expressed on different blood cells. By comparing CD molecules expressed on AML cells with the ones normally expressed on healthy progenitor or differentiating cells, disease specific phenotypes can be detected. Since many years, immunophenotyping is an easy to apply, well established technique and commonly used in clinical practice. Today, it is not only a valuable diagnostic and classification tool in AML but immunophenotyping was also shown to have prognostic significance and is increasingly used for minimal residual disease detection during further disease course.[43]

Although its exact function is unknown, the hematopoietic progenitor cell antigen CD34 functions as cell-cell adhesion factor in bone marrow cells, possibly mediating the attachment of human HSCs to bone marrow stroma. The co-expression of CD34 with CD38 indicates lineage dedication and is found on over 90% of CD34+ cells.[44] The relatively small subset of CD34+/CD38- cells in bone marrow contains the majority of a highly primitive cell population

that is assumed to maintain hematopoiesis.[45] These HSCs have the potential of self-renewal and can also differentiate into all types of mature blood cells.[46] CD34+/CD38- cells are able to initiate re-transplantable human hematopoiesis when transplanted into Non-Obese Diabetic/ Severe Combined Immunodeficiency (NOD/SCID) mice.[47]

Leukemia Initiating Cells

Similar to normal hematopoiesis, AML is believed to be hierarchically structured.[48] A likewise small subset of leukemic cells shows the immature CD34+/CD38- phenotype.[48,49] Interestingly, this population is able to initiate human leukemia not only in one, but also in higher generations of NOD/SCID mice. This led to the current understanding that most AML clones arise from a primitive cell population, named leukemia initiating or leukemic stem cell (LIC), that itself originates from a normal HSC and shares its CD34+/CD38- phenotype.[48,50] In later studies, both healthy HSCs and LICs were described to be CD44+ and HLA-DR-.[51-53] Other studies indicated that LICs may not only share some phenotype characteristics with normal HSCs but also seem to differ substantially from them: the surface expression of CD96 or CD123 as well as the absence of CD117 has been suggested to have the ability to distinguish LICs from healthy HSCs.[51,54-57] However, none of these markers has been proven the ability to infallibly define the LIC population. Similarly, although LICs and HSCs may share some surface marker expressions, LICs show significantly different gene and protein expression than healthy HSCs or AML bulk cells[58,59] and LIC gene expression signatures were already shown to impact on survival of AML patients.[60] These surface proteins expressed on leukemic CD34+/CD38- cells but not healthy HSCs would not only offer the potential to further characterize the LIC population but may also offer new therapeutic avenues. Monoclonal antibodies against CD96 to selectively eradicate LICs in *vitro* use before autologous HSCT[61] or *in vivo* as target structure for Fc-engineered mini-antibodies[62] are under development. The monoclonal CD123 antibody CSL362 exhibits cell mediated toxicity against LICs and AML bulk cells[63] and is currently tested in a phase I trial (Clinical

Trials.gov identifier: NCT01632852) on high risk AML patients not eligible for HSCT. CD123 chimeric antigen receptors (CARS) are also being developed and tested in mice trials.[64] Aurora kinase A is one of the afore mentioned proteins that is believed to be distinctively expressed in LICs compared to healthy HSCs,[65] and the Aurora kinase A inhibitor MLN8237 impairs self-renewal, inhibits proliferation and leads to higher apoptosis rates in AML cells.[65] After treatment of NOD/SCID mice resulted in impaired cell engraftment and longer mice survival, the orally available substance now entered clinical trials in humans. Further potential lies in therapeutically influencing reversible epigenetic changes such as DNA-methylation or histone deacetylation in LICs, e. g. with the EZH2-inhibitor 3-Deazaneplanocin A which promotes apoptosis in LICs but not in healthy HSCs.[66,67]

Compared to the majority of circulating, proliferating leukemic blasts or "bulk cells", LICs are relatively resistant to chemotherapeutic agents.[68] One reason might be that a large subset of CD34+/CD38- cells are metabolically inactive, resting in the G0 cell cycle phase.[69] Some studies revealed a shorter survival and higher relapse incidences in AML patients with a high CD34+/CD38- burden at diagnosis who were treated with chemotherapy.[51,70-72] LICs were also described to be less immunogenic than AML bulk cells:[73] *In vitro* data showed lower lymphocyte proliferation against the CD38- population, as well as decreased IL-2 and IFN-γ secretion in the CD34+/CD38- cells. Reasons might be a lower major immune response molecule expression on the CD34+/CD38- cell population.[71] This supports the assumption that a higher LIC population burden at diagnosis may also impact adversely on patients' outcome after allogeneic HSCT. However, until today no study assessed the LIC population burden related outcomes in AML patients receiving an allogeneic HSCT, a procedure for which the therapeutic impact also relies on cell-derived immunologic effects.

Objectives of the here presented study

1. To analyze the prognostic impact of the bone marrow CD34+/CD38- LIC population at diagnosis in AML patients who received an allogeneic HSCT for consolidation therapy.
2. To uncover possible associations of the bone marrow CD34+/CD38- LIC population at diagnosis with other clinical and molecular characteristics and prognostic factors in AML.
3. To evaluate whether the bone marrow CD34+/CD38- LIC population at diagnosis refines todays risk stratification in AML.

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RESEARCH ARTICLE

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Prognostic impact of the CD34+/CD38− cell burden in patients with acute myeloid leukemia receiving allogeneic stem cell transplantation

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Abstract

In acute myeloid leukemia (AML), leukemia-initiating cells exist within the CD34+/CD38− cell compartment. They are assumed to be more resistant to chemotherapy, enriched in minimal residual disease cell populations, and responsible for relapse. Here we evaluated clinical and biological associations and the prognostic impact of a high diagnostic CD34+/CD38− cell burden in 169 AML patients receiving an allogeneic stem cell transplantation in complete remission. Here, the therapeutic approach is mainly based on immunological graft-versus-leukemia effects. Percentage of bone marrow CD34+/CD38− cell burden at diagnosis was measured using flow cytometry and was highly variable (median 0.5%, range 0%–89% of all mononuclear cells). A high CD34+/CD38− cell burden at diagnosis associated with worse genetic risk and secondary AML. Patients with a high CD34+/CD38− cell burden had shorter relapse-free and overall survival which may be mediated by residual leukemia-initiating cells in the CD34+/CD38− cell population, escaping the graft-versus-leukemia effect after allogeneic transplantation. Evaluating the CD34+/CD38− cell burden at diagnosis may help to identify patients at high risk of relapse after allogeneic transplantation. Further studies to understand leukemia-initiating cell biology and develop targeting therapies to improve outcomes of AML patients are needed.

1 | INTRODUCTION

Hematopoietic stem cells (HSCs) are found in the bone marrow (BM) and physiologically give rise to all blood cells.[1] The phenotype of HSCs is not well defined, but HSCs have been suggested to be part of the primitive CD34+/CD38− cell population.[2] A high amount of the CD34+/CD38− cells seem to rest in the G0 cell cycle phase,[3] and are able to initiate retransplantable hematopoiesis in animal models.[4]

Acute myeloid leukemia (AML) is a disease originating from the clonal expansion of HSCs or early progenitor cells that have lost the ability to mature.[5] Most researchers agree that similar to the physiological HSCs, AML-initiating cells—often termed leukemia-initiating cells (LICs)—also exist within the CD34+/CD38− stem cell compartment.[6–9] LICs—opposed to the majority of circulating, proliferating leukemic blasts or "bulk cells"—are postulated to survive chemotherapy as minimal residual disease (MRD) and cause AML relapse; their nonproliferative state might be one reason for resistance to chemotherapy.[10–14] When transplanted into NOD/SCID mice, CD34+/CD38− cells of AML patients were able to initiate leukemia and growth of tumors histologically similar to the human donor's neoplasm.[7–15]

*Presented in part at the Annual Meeting 2014 of The European Hematology Association (EHA) and the Annual Meeting 2015 of the European Bone Marrow Transplantation (EBMT) and published in abstract form.

Hematopoietic stem cell transplantation (HSCT) offers a curative treatment option for AML patients if a suitable donor is available. After achievement of complete remission (CR), HSCT is an established form of consolidation therapy for patients at high risk of relapse.[16] The therapeutic effects of HSCT are mainly based on an immunologic graft-versus-leukemia (GvL) reaction.[17]

Previous studies have suggested that a high burden of LICs at diagnosis of AML patients treated with chemotherapy increased their relapse probability and associated with inferior outcomes.[14,18–20] However, it is at present unknown whether a high burden of LICs has a similar prognostic significance in patients undergoing HSCT. As data support the assumption that LICs are also less immunogenic than the leukemic bulk cells,[21] evaluation of the pretreatment LIC or the CD34+/CD38− cell burden in AML patients after allogeneic HSCT might provide important biological and clinical information. The objective of this study was to determine the prognostic significance of the CD34+/CD38− cell burden at diagnosis in patients undergoing HSCT and to analyze associations between the CD34+/CD38− cell burden and clinical, cytogenetic, and molecular characteristics to provide further biological insights.

2 | PATIENTS AND METHODS

2.1 | Patients and treatment

We analyzed 169 adult AML patients who received HSCT in CR at the University of Leipzig between June 2001 and July 2013, and for whom pretreatment BM aspirate material for flow cytometry analysis was available. All patients received standard cytarabine-based protocol chemotherapy and were transplanted in the first or second CR (for details, please see Supporting Information). Written informed consent for participation in these studies was obtained in accordance with the Declaration of Helsinki.

Forty-nine (29%) patients received myeloablative conditioning (MAC), which consisted of cyclophosphamide 60 mg/kg body weight for 2 days and 12 Gy total body irradiation (TBI),[22] whereas 120 (71%) patients received nonmyeloablative conditioning (NMA), which contained fludarabine 30 mg/m^2 for 3 days followed by 2 Gy TBI.[23,24] All patients received granulocyte colony stimulating factor-mobilized peripheral blood stem cells on day 0. Reasons for NMA, as opposed to MAC conditioning, were age (patients over 50 years if receiving unrelated HSCT [$n = 102$] and patients over 55 years if receiving related HSCT [$n = 17$]) and previous autologous HSCT ($n = 1$).

Prior to HSCT, all patients were treated according to age-dependent chemotherapy protocols (under or over 60 years); for details, please see Supporting Information. Patients' characteristics are shown in Table 1 and Supporting Information, Table S1. At HSCT, patients had a median age of 62 years (range 19–75) and according to protocols, patients receiving MAC-HSCT were significantly younger than patients receiving NMA-HSCT (MAC-HSCT, median 40, range 19–55; NMA-HSCT, median 66, range: 47–75 years; $P < .001$). Survival analyses according to conditioning regimes are provided in Supporting Information, Figure S1 and Table S2.

At the time of HSCT, 83% of all patients (84% in MAC-HSCT and 83% in NMA-HSCT) were in their first and 17% (16% in MAC-HSCT and 18% in NMA-HSCT) were in second CR (for CR definition, see Supporting Information). Donors were human leukocyte antigen (HLA)-matched related in 23% of cases (39% in MAC-HSCT and 14% in NMA-HSCT), while 54% (53% in MAC-HSCT and 58% in NMA-HSCT) were HLA-matched unrelated and 23% (8% in MAC-HSCT and 28% in NMA-HSCT) were unrelated with at least one antigen mismatch.

2.2 | Cytogenetics and molecular markers

Pretreatment BM cytogenetic analyses were performed centrally in our institution. The presence of internal tandem duplication in the *FLT3* gene (*FLT3*-ITD), mutations in the *FLT3* tyrosine kinase domain (*FLT3*-TKD), and in the *NPM1* and *CEBPA* genes were determined as described previously.[25–27]

2.3 | Flow cytometry

For all 169 patients, mononuclear BM cells were assessed for surface expression of CD34, CD38, and CD34/CD38 at diagnosis. For details, please see Supporting Information.

2.4 | Definition of clinical end points and statistical analysis

Statistical analyses were performed using the R statistical software platform (version 3.0.2). Utilizing the "OptimalCutpoints" package, an optimal cutoff of 6% was identified that divided the cohort into patients with a high or a low CD34+/CD38− cell burden.

Overall survival (OS) was calculated from HSCT until death from any cause and relapse-free survival (RFS) was calculated from HSCT to relapse or death from any cause.

Associations of CD34+/CD38− burden with baseline clinical, demographic, and molecular features were compared using the Kruskal–Wallis test and Fisher's exact test for continuous and categorical variables, respectively. For time-to-event analyses, survival estimates were calculated using the Kaplan–Meier method. Groups were compared with the log-rank test. Multivariable analysis is described in Supporting Information.

3 | RESULTS

3.1 | Associations of CD34+/CD38− cell burden in BM at diagnosis with genetic and clinical characteristics

The CD34+/CD38− cell burden at diagnosis was highly variable (median 0.5%, range 0%–89% of all mononuclear cells). There was no difference in the CD34+/CD38− cell burden between younger (<60 years) and older (≥60 years) patients at diagnosis ($P = .14$, Figure 1A). However, while there were no differences between age groups in the European LeukemiaNet (ELN),[28] Favorable, Intermediate-I, or Intermediate-II Genetic Groups (Figure 1B–D), older patients in the

TABLE 1 Clinical characteristics of AML patients treated with HSCT according to CD34+/CD38− cell burden at diagnosis (< 6% vs ≥6%)

Characteristics	All patients (*n* = 169)	Low CD34+/CD38− cell burden (*n* = 144)	High CD34+CD38− cell burden (*n* = 25)	*P*
Age at HSCT, years				.14
Median	62	61	63	
Range	19–75	19–75	30–74	
Sex, *n* (%)				.67
Male	85 (50)	71 (49)	14 (56)	
Female	84 (50)	73 (51)	11 (44)	
Hemoglobin, g/dL				.45
Median	8.6	8.6	9.4	
Range	4.5–15.7	4.5–15.7	5.3–13.3	
Platelet count, $\times 10^9$/L				.04
Median	63	74	40	
Range	2–327	2–327	13–178	
WBC count, $\times 10^9$/L				.07
Median	7.9	7.5	35.5	
Range	0.7–385	0.7–385	1.1–295	
Percentage of blood blasts, %				.11
Median	26	22	47	
Range	0–98	0–98	2–97	
Percentage of BM blasts, %				.83
Median	56	56	60	
Range	0–95	0–95	0–95	
Karyotype, *n* (%)				.39
Abnormal	84 (52)	69 (50)	15 (60)	
Normal	79 (49)	69 (50)	10 (40)	
ELN genetic group, *n* (%)				.001
Favorable	42 (30)	41 (32)	1 (5)	
Intermediate-I	39 (26)	33 (25)	6 (29)	
Intermediate-II	32 (21)	30 (23)	2 (10)	
Adverse	38 (25)	26 (20)	12 (57)	
Disease origin, *n* (%)				.009
De novo	116 (69)	105 (73)	11 (44)	
Secondary	53 (31)	39 (27)	14 (56)	
MDS	32	25	7	
MPN	10	6	4	
Solid tumor	11	8	3	
***NPM1*, *n* (%)**				.19
Wild-type	109 (74)	90 (71)	19 (86)	
Mutated	39 (26)	36 (29)	3 (14)	

(*continued*)

TABLE 1 (continued)

Characteristics	All patients (n = 169)	Low CD34+/CD38− cell burden (n = 144)	High CD34+CD38− cell burden (n = 25)	P
***FLT3-ITD*, *n* (%)**				.77
Absent	114 (78)	99 (79)	15 (75)	
Present	32 (22)	27 (21)	5 (25)	
***CEBPA*, *n* (%)**				.13
Wild-type	114 (85)	98 (83)	16 (100)	
Mutated	20 (15)	20 (17)	0 (0)	

Abbreviations: BM, bone marrow; ELN, European LeukemiaNet; FLT3-ITD, internal tandem duplication of the FLT3 gene; HSCT, hematopoietic stem cell transplantation; MAC, myeloablative; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasia; NMA, nonmyeloablative; WBC, white blood cell.

ELN Adverse Genetic Group had a trend for a higher CD34+/CD38− cell burden at diagnosis than younger patients (P = .10, median 2.7% vs 1%, Figure 1E).

In further analyses, we used a 6% cutoff to divide the patients according to their CD34+/CD38− cell burden at diagnosis into low [<6%, n = 144 (85%)] and high [≥6%, n = 25 (15%)] burden groups. Subgroup analyses restricted to patients receiving HSCT in the first CR are shown in Supporting Information, Table S3 and Figure S5.

At diagnosis, a high CD34+/CD38− cell burden associated with lower platelet counts (P = .04), and, by trend, higher white blood cell (WBC) counts (P = .07). Patients developing AML as a secondary disease following myelodysplastic syndrome, myeloproliferative neoplasia, or solid tumors were more likely to have a high CD34+/CD38− cell burden at diagnosis than patients with *de novo* AML (P = .009). Patients with a high CD34+/CD38− cell burden were more likely to have a complex karyotype (≥3 cytogenetic abnormalities,[28] P = .02), monosomy 5 or deletion of 5q (P = .004) or to have a monosomal karyotype[29] (P = .004). Among the ELN Genetic Groups, patients with high CD34+/CD38− cell burden at diagnosis were less often classified in the Favorable (5% vs 32%) and more often in the Adverse (57% vs 20%) Genetic Group (P = .001, Table 1). Furthermore, none of the patients with a high CD34+/CD38− cell burden at diagnosis harbored a *CEBPA* mutation (P = .13).

3.2 | Prognostic value of CD34+/CD38− cell burden at diagnosis

A high CD34+/CD38− cell burden at diagnosis associated with shorter RFS (P < .001, Figure 2A) and OS (P = .005, Figure 2B). When the distinct HSCT-conditioning protocols were regarded separately, we

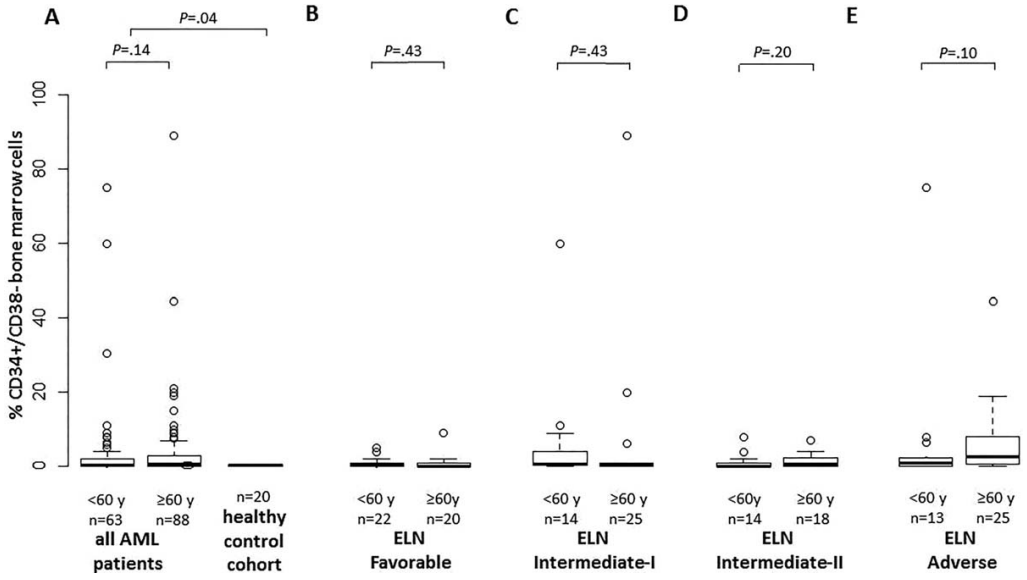

FIGURE 1 CD34+/CD38− cell burden according to age at diagnosis (<60 years vs ≥60 years). (A) All patients (n = 169) *versus* healthy subjects (n = 20). (B) Favorable ELN genetic risk. (C) Intermediate-I ELN genetic risk. (D) Intermediate-II ELN genetic risk. (E) Adverse ELN genetic risk

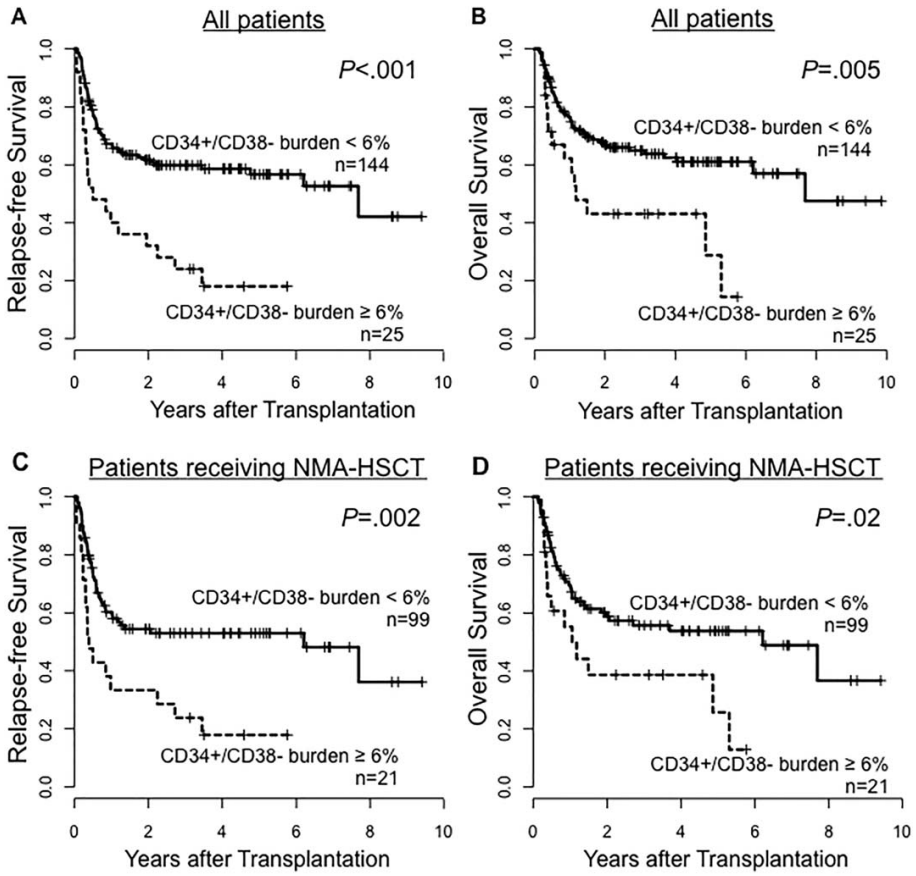

FIGURE 2 Outcome of HSCT-treated AML patients according to the CD34+/CD38− cell burden at diagnosis (<6% vs ≥6%). (A) Relapse-free survival and (B) overall survival for all patients ($n = 169$) and (C) relapse-free survival and (D) overall survival for patients receiving NMA-HSCT ($n = 120$)

observed shorter RFS ($P = .002$, Figure 2C) and OS ($P = .02$, Figure 2D) for patients with a high CD34+/CD38− cell burden treated with NMA-HSCT. In the group of MAC-HSCT-treated patients, only four had a high CD34+/CD38− cell burden at diagnosis preventing further subanalyses of the MAC-HSCT-treated patients. Three years after transplantation, 65% of patients with a low diagnostic CD34+/CD38− cell burden were alive (including 56% of NMA-HSCT-treated patients) and 60% relapse-free (including 53% of NMA-HSCT-treated patients). In contrast, in the group with a high CD34+/CD38− cell burden, only 43% of patients were alive (including 39% of NMA-HSCT-treated patients) and only 24% (including 24% of NMA-HSCT-treated patients) remained relapse-free after 3 years. The CD34+/CD38− cell burden at diagnosis did also impact on RFS and OS when we restricted our analysis to patients with normal karyotype or *de novo* AML (Supporting Information, Figures S2 and S3). Furthermore, in patients with ELN Favorable or Intermediate genetic risk who had a high CD34+/CD38− cell burden at diagnosis, we observed a trend for shorter RFS ($P = .06$) and comparable OS ($P = .11$) to patients within the ELN Adverse Genetic Group (Supporting Information, Figure S8).

In multivariable analysis (Table 2), a high CD34+/CD38− cell burden at diagnosis remained significantly associated with shorter RFS ($P < .001$) and shorter OS ($P = .04$) after adjustment for age at HSCT. The risk of death or an event was about twice as high in patients with a high diagnostic CD34+/CD38− cell burden compared with that of patients with a low CD34+/CD38− cell burden.

4 | DISCUSSION

Similar to normal hematopoiesis, AML cells are thought to emerge from primitive LICs with the ability of unlimited self-renewal.[15,30] The observation that leukemic CD34+/CD38− cells are able to serially transplant AML in NOD/SCID mice[6,14,15] led to the assumption that the CD34+/CD38− BM compartment harbors the LIC population.[30] AML bulk cells are often efficiently eradicated by chemotherapeutic agents. In contrast, LICs show resistance to chemotherapeutic agents.[31,32] LICs exist in a quiescent state within a stem cell niche and have slow dividing properties.[33] Among other biological features, this

TABLE 2 Multivariable outcome analyses of 169 AML patients treated with HSCT according to the CD34+/CD38− burden at diagnosis

	Relapse-free survival			Overall survival		
Variable	HR[a]	95% CI	*P*	HR[a]	95% CI	*P*
Age at the time of HSCT	1.03	1.01–1.05	.007	1.05	1.02–1.08	<.001
CD34+/CD38− cell burden at diagnosis (≥6% vs <6%)	2.44	1.46–4.08	<.001	1.86	1.05–3.37	.04

Abbreviations: CI, confidence interval; HSCT, hematopoietic cell transplantation; HR, hazard ratio; MAC, myeloablative; NMA, nonmyeloablative.
[a]HR <1 (>1) indicate lower (higher) risk for an event for the first category listed for the dichotomous variables and for the higher values of the continuous variables.
Variables considered in the models were those significant at $\alpha = 0.20$ in univariable analyses. Variables considered were sex, disease origin (de novo vs secondary), ELN classification, platelet count at diagnosis, blast count in bone marrow at diagnosis, CD34+/CD38− burden at diagnosis, age at HSCT, disease status at HSCT (first vs second CR), HLA match (antigen match vs mismatch), and HLA donor type (related vs unrelated).

quiescence and the increased expression of multidrug resistance genes may explain the occurrence of relapse after cytotoxic therapy.[21] Furthermore, there is growing evidence that leukemic CD34+/CD38− cells are also less immunogenic than AML bulk cells. Costello et al.[21] showed reduced immunogenicity in vitro with lower lymphocyte proliferation against a CD38− population and decreased IL-2 and IFN-γ secretion. Reasons for this observation might be a lower expression of major immune response molecules in the CD34+/CD38− cell population. These observations led us to explore the outcomes as well as biological and clinical characteristics associated with the CD34+/CD38− cell burden at diagnosis in AML patients undergoing HSCT.

While three previous studies did not find associations of a high CD34+/CD38− cell burden with cytogenetics,[18,19,34] which may be related to the varying numbers and characteristics of patients analyzed, we found that patients with a high CD34+/CD38− cell burden were more likely to have poor-risk cytogenetics. In line with these findings, we also observed an unequal distribution of patients with high and low CD34+/CD38− cell burden in the four ELN Genetic Groups, with patients with a high CD34+/CD38− cell burden being least often classified in the Favorable and most frequently in the Adverse Group (Table 1 and Supporting Information, Table S1). Another study described an association of a CD34+/CD38−/ALDH^{high} LIC phenotype cell burden with poor-risk genetics (ELN adverse genetic risk, monosomal, or complex karyotypes) in a cohort of 98 patients.[35] Patients with a high CD34+/CD38− cell burden were also more likely to have secondary AML, which was also found for the patients harboring a CD34+/CD38−/ALDH^{high} LIC phenotype.[35] Thus, the size of the LIC population might be interconnected and contribute to the known adverse outcome of AML with adverse cytogenetic risk, for example, monosomal or complex karyotypes and secondary AML. Furthermore, none of the patients with a high CD34+/CD38− cell burden at diagnosis was *CEBPA*-mutated compared to 17% of patients with a low CD34+/CD38− cell burden. We did not observe significant associations of a high CD34+/CD38− cell burden with the presence of *FLT3*-ITD or *NPM1* mutations in the entire cohort. However, when we restricted our analysis to patients receiving HSCT in the first CR, none of the patients with a high CD34+/CD38− cell burden at diagnosis were *NPM1* mutated ($P = .01$, Supporting Information, Table S3). A small number of studies analyzed the LIC population burden at diagnosis in the context of molecular markers and, similar to our study, none found an association with the presence of *FLT3*-ITD.[14,18,19,34] Whereas Vergez et al.[19] did not find an association of the presence of *NPM1* mutations with the LIC population burden (defined by CD34 + CD38^{low}/−CD123+), Gerber et al.[35] described a decreased frequency of *NPM1* mutations in an LIC phenotype defined by CD34+/CD38−/ALDH^{high}. It is known that *CEBPA* mutations activate self-renewal capacity in committed myeloid progenitor cells,[36] resulting in more mature AML phenotypes, and that *NPM1* mutations occur in more mature CD34− AML cells.[37] As the presence of mutations in both genes impact positively on survival in cytogenetically normal AML,[38] the observed associations may indicate an important biological interaction between *CEBPA* or *NPM1* mutations and the LIC burden at diagnosis in AML patients.

We were able to show that a high CD34+/CD38− cell burden at diagnosis associated with shorter RFS and OS after HSCT in CR. In multivariable analysis, the CD34+/CD38− cell burden retained its prognostic value, independently of other known prognostic factors. Some studies demonstrated that a high burden of LICs at AML diagnosis increased the relapse probability after chemotherapy and associated with worse outcomes in different patient cohorts.[13,18–20,35] In general, these studies described a wide range of diagnostic BM CD34+/CD38− cells, from 0.01% up to 71%, in AML patients, similar to the findings in our study. Van Rhenen et al.[14] found the diagnostic CD34+/CD38− cell burden of AML patients associated with a higher MRD frequency evaluated by flow cytometry after chemotherapy, shorter OS, RFS, and disease-free survival (DFS). Hwang et al.[34] described a higher CD34+/CD38− cell burden at diagnosis in patients who did not achieve a CR after one course of chemotherapy. Khan et al.[20] showed lower CR rates and shorter OS for patients over 60 years of age with a higher diagnostic CD34+/CD38− cell burden in blood, but not in BM. Other studies further characterized the analyzed LIC population. Vergez et al.[19] demonstrated that a high CD34 + CD38^{low}/−CD123+ cell burden associated with a lower CR rate, shorter DFS, and shorter OS. Wang et al.[18] performed flow cytometry on FISH-preselected blasts in AML with abnormal karyotype and demonstrated a shorter OS and RFS and higher relapse rates in patients with a high FISH+/CD34+/CD38− cell burden at diagnosis. In these studies, the chosen cutoffs to define a high LIC burden were those with the most significant outcome impact and ranged from 1% to 15%.[14,18–20] This stands in line with our finding with an optimal

cutoff at 6% but also significant outcome differences using a 2%, 7.5%, or 10% cutoff (Supporting Information, Figure S4). Gerber et al.[35] showed lower CR rates and shorter EFS and OS in patients with a more immature LIC phenotype (CD34+/CD38−/ALDH^{high} vs CD34+/CD38−/ALDH^{intermediate} vs CD34−). However, within these studies, only 16%–47% of all patients and only younger individuals underwent HSCT, and none of the studies separately investigated the outcome of a larger cohort of HSCT-treated patients, for whom the GvL effect is thought to provide a continuous impact on residual disease. To our knowledge, this study is the first to show an adverse outcome of patients with a high LIC-containing cell population at diagnosis in a larger cohort treated with HSCT as consolidation therapy. Furthermore, the majority of AML patients we analyzed received an NMA-conditioning protocol,[23,39] for which the therapeutic effect is nearly exclusively based on the GvL effect. The aforementioned reduced immunogenicity of LICs observed by Costello et al.[21] is supported by our finding that HSCT may not be able to fully overcome the described poor prognosis of a high LIC burden at diagnosis. Clinically, not unlike cytotoxic agents, the GvL effect may primarily impact on AML bulk cells, and LICs within their BM niche may at least partly be able to evade the GvL effect. This observation helps to deepen our understanding why after HSCT in CR, some AML patients remain in remission while others do not.

We also compared the CD34+/CD38− cell burden in AML patients at diagnosis to the CD34+/CD38− BM cell counts during disease course (in CR before HSCT, at day 28 after HSCT, at relapse) and to that of 20 healthy individuals (for details, see Supporting Information). The CD34+/CD38− cell counts of AML patients in CR were comparable to or even lower than the CD34+/CD38− cell count in the healthy cohort and no significant difference was observed between patients with a high or a low CD34+/CD38− cell burden at diagnosis. Furthermore, the CD34+/CD38− cell counts were higher during AML relapse and comparable to the CD34+/CD38− cell burden at diagnosis. For details, see Supporting Information, Figure S9. However, as healthy HSCs also show the CD34+/CD38− phenotype, this population alone does not seem to present a suitable marker for risk assessment in CR or MRD detection.

A high CD34+/CD38− cell burden at AML diagnosis independently associated with worse outcomes in patients undergoing HSCT suggesting that determination of the CD34+/CD38− cell burden at diagnosis may provide a simple and widely available method to improve risk stratification in AML patients. This may help to identify patients in need of closer remission monitoring and possibly adjustment of therapeutic approaches, for example, tapering of immunosuppressant agents after HSCT. However, prospective studies to validate our findings are needed.

Given the inferior outcomes of AML patients with a high CD34+/CD38− cell burden at diagnosis that we and others[14,18–20,34,35] observed, regardless of HSCT as a consolidating therapy, new strategies to target LICs may improve survival. For example, the ability to target the CD34+/CD38− cell population was shown for Gemtuzumab ozogamicin,[34] and in vitro combination with tipifarnib suggested synergistic effects, especially on the LIC population.[40] Even though some evidence points to an intraindividual and interindividual heterogenic LIC phenotype,[35] some surface markers such as CD123,[34,41,42] CD96,[43] or CD117[44] may be able to discriminate between healthy HSCs and AML LICs, and ways to therapeutically exploit these phenotype differences are under investigation.[45,46] As CD123 seems to be expressed on LICs rather than HSCs, another promising therapy are T-cells expressing CD123-specific chimeric antigen receptors with high effector activity against AML cell lines and patient samples in vitro without affecting granulocyte or erythroid colonies.[47] Further potential therapeutic targets may be identified from genes and proteins differentially expressed in LICs compared to AML bulk cells or healthy HSCs.[9,48–50] Combining therapeutic approaches derived from these studies with chemotherapy and/or HSCT may help to improve outcomes—especially for those patients who have a high diagnostic CD34+/CD38− cell burden.

In conclusion, our data demonstrate that the negative prognostic impact of a high CD34+/CD38− cell burden at diagnosis seems not to be easily overcome by the GvL effect after HSCT in AML patients. In multivariable analysis, a high CD34+/CD38− cell burden at diagnosis was an independent factor for shorter RFS and OS, likely mediated by LICs within the CD34+/CD38− cell population escaping the GvL effects of HSCT. However, HSCT versus non-HSCT studies will be needed to evaluate whether patients with a high CD34+/CD38− cell burden at diagnosis might benefit from a HSCT as consolidation therapy, despite their worse outcome than patients with a low burden at diagnosis. Determination of the CD34+/CD38− cell burden at AML diagnosis may help to improve risk stratification, adjust disease monitoring and treatment, especially that it is widely available and relatively inexpensive. Finally, novel therapeutic agents targeting AML LICs within the CD34+/CD38− cell population may help to improve outcomes of these patients.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest with the contents of this article.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

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Anlagen / Supplemental Material

SUPPLEMENTARY DATA

M. Jentzsch *et al.*: **Prognostic Impact of the CD34+/CD38- Cell Burden in Patients with Acute Myeloid Leukemia Receiving Allogeneic Stem Cell Transplantation**

Further genetic and HSCT related information

Additional cytogenetic and biological information and hematopoietic stem cell transplantation (HSCT) related information are shown in Supplementary Table SI.

Treatment protocols

Sixty-eight (91%) patients diagnosed with acute myeloid leukemia (AML) at the age of 60 years or younger received induction and consolidation therapy according to the AML 2002 study (#061, ClinicalTrials.gov Identifier: NCT01414231), six (8%) patients received chemotherapy within the PKC412 protocol (ClinicalTrials.gov Identifier: NCT00111345) and one patient (1%) was diagnosed with AML as a child and treated within the AML BFM-2014 study (ClinicalTrials.gov Identifier: NCT00651261).

Among AML patients older than 60 years, 91 (97%) were treated within the AML 2004 (#069, ClinicalTrials.gov Identifier: NCT01497002) study and 3 (3%) patients were treated within the AML 2004 (#069, ClinicalTrials.gov Identifier: NCT01497002) protocol and additionally received demethylating agents (azacitidine).

Patients harboring a core-binding factor AML received an allogeneic HSCT in second complete remission (CR) or according to patients' choice in first CR if a suitable donor was available.

Definition of complete remission

Complete remission (CR) was defined as the presence of <5% of blasts in bone marrow (BM), neutrophils >1.0 x 10^9/L, platelets > 100 x 10^9/L, absence of blasts with Auer rods,

independence of blood transfusion and no extramedullary disease [1]. The presence of CR was confirmed within 28 days prior to HSCT by BM and peripheral blood analysis.

Prevention of graft-versus-host disease

Prevention of graft-versus-host disease (GvHD) was different according to the two conditioning regimes used. All patients receiving myeloablative conditioning (MAC) were treated with cyclosporine A (CyA), starting intravenously with 5 mg/kg body weight (BW) in two daily doses from day -1. Blood levels of CyA were measured from day 0 and doses were adjusted for target levels of 200 ng/ml. Patients also received methotrexate 15 mg intravenously on days +1, +3, +6 and +11 after HSCT. Furthermore, patients with an unrelated donor additionally received *in vivo* T-cell depletion with thymoglobulin 2 mg/kg BW per day on days -3 to -1.

All patients with non-myeloablative conditioning (NMA) received a starting dose of 5 mg/kg BW CyA in two daily doses from day -1. Blood levels of CyA were measured from day 0 and doses were adjusted for target levels of 200 ng/ml. Additionally, patients with NMA conditioning received mycophenolate mofetil (MMF) 3 g per day in three daily doses if receiving unrelated HSCT or 2 g per day in two daily doses if receiving related HSCT. CyA was reduced starting on day +84 or day +180 following related or unrelated HSCT, respectively, and MMF was stopped at day +28 following related HSCT and tapered from days +40 to +96 following unrelated HSCT.

For all patients after MAC- or NMA-HSCT, immunosuppression was prolonged or extended with systemic steroids in cases of GvHD (grade > 2 according to Glucksberg grading system [2]) or rapidly reduced in patients who relapsed (≥ 5% blasts in BM).

Incidence of GvHD

Patients were evaluated for incidence of acute GvHD (aGvHD) and chronic GvHD (cGvHD), using established criteria of the Glucksberg grading system [2]. Requirement for aGvHD was

engraftment while requirements for cGvHD was engraftment and survival for at least 100 days after HSCT.

Acute GvHD (grade 2-4) appeared in 42 patients (29%), whereas 76 (61%) patients had cGvHD, including 20 (16%) patients with limited and 56 (45%) with extended cGvHD. There was no difference in aGvHD development between patients with a high and those with a low CD34+/CD38- cell burden at diagnosis as well as cGvHD development in patients surviving more than 100 days after HSCT (*P*=.44, Supplementary Table SI).

Cytogenetic Analysis

Pretreatment BM cytogenetic analyses were performed centrally in our institution using standard banding techniques. In cases were no metaphases could be obtained, fluorescence *in-situ* hybridization (FISH) was used to screen for recurrent abnormalities (i.e. del5/5q, del7/7q, trisomy 8, inv(3), abn(p53), abn11q23, t(8;21), inv(16) and t(15;17); [n=5]).

Flow cytometry and evaluation of CD34+/CD38- cell count

EDTA-anticoagulated fresh BM aspirates were obtained from all 169 patients at AML diagnosis prior to beginning of chemotherapy. In all samples, surface marker expression was measured with the FACSCalibur flow cytometer and analyzed using the CELLQUEST software (Becton Dickinson, Heidelberg, Germany). Samples were stored at room temperature and processed within 12 hours. One-hundred microliters of BM aspirates were incubated for 15 minutes with phycoerythrin-Cy7 (PE-Cy7, CD38) and allophycocyanin (APC, CD34) labeled monoclonal antibodies. After erythrocyte lysis, samples were washed in 1 ml of phosphate buffered saline (PBS) solution fixed in 500 µl PBS Acid with 1% formaldehyde; 10,000 cells per sample were analyzed.

Survival of MAC- or NMA-HSCT treated AML patients

The whole patient cohort had a 5-year relapse-free survival (RFS) rate of 50% [confidence interval (CI) 42-59%, Supplementary Figure S1A] and a 5-year overall survival (OS) rate of 57% (CI 49-66%, Supplementary Figure S1B). Cumulative Incidence of Relapse (CIR) at 5 years after HSCT was 36% and the competing event non-relapse mortality (NRM) at 5 years was 14%. RFS, OS, CIR and NRM at 2 and 3 years after HSCT are shown in Supplementary Table SII. Median follow-up after HSCT was 3.4 years (range, 0.3-9.9 years).

Patients receiving MAC-HSCT had a 5-year RFS rate of 60% and a 5-year OS rate of 75% (Supplementary Figures S1C, S1D). Furthermore, a NRM affected 4% and CIR was 36% in MAC-HSCT treated patients (Supplementary Table SII). Survival and relapse rates are in line with those reported in the existing literature [3-7]. Within the older and in part heavily pretreated NMA-HSCT cohort, we observed a 5-year RFS rate of 46% and a 5-year OS rate of 49% (Supplementary Figures S1E, S1F). NRM was 18% of NMA-HSCT treated patients and CIR was 36% of patients. (Supplementary Table SII). This data is comparable with the existing literature reporting on similar NMA protocols [7-9].

Compared with patients receiving NMA-HSCT, those treated with MAC-HSCT had a significantly longer RFS (P=.01) and OS (P<.001). There was no significant difference in relapse rates between the two conditioning regimes (P=.59).

Multivariable analysis

We constructed a multivariable proportional hazard model for OS and RFS to evaluate the impact of the CD34+/CD38- cell burden at diagnosis by adjusting for other variables.

In addition to the CD34+/CD38- cell burden at diagnosis (low vs high), the following variables were considered for multivariable analyses: sex, hemoglobin count, platelet count, white blood cell count, blast percentages in peripheral blood and BM at diagnosis, AML type (*de novo* vs secondary), European LeukemiaNet (ELN) Genetic Groups, mutation status of the tyrosine

kinase domain of the *FLT3* gene (*FLT3*-TKD), age at HSCT, disease status at HSCT (CR1 vs CR2), cytomegalovirus (CMV) status of recipient and donor (high risk [+/-] vs all others), HLA match (antigen match vs mismatch), HLA donor type (related vs unrelated) and sex of the donor. Of these, variables significant at α=.20 in univariable analyses were considered for multivariable analyses. For OS endpoint, these variables were sex, disease origin (*de novo* vs secondary), ELN classification, platelet count at diagnosis, blast count in bone marrow at diagnosis, CD34+/CD38- burden at diagnosis, age at HSCT, HLA match (antigen match vs mismatch) and HLA donor type (related vs unrelated) while for RFS endpoint, these variables were disease origin (*de novo* vs secondary), ELN classification, platelet count at diagnosis, blast count in bone marrow at diagnosis, CD34+/CD38- burden at diagnosis, age at HSCT, disease status at HSCT (first vs second CR) and HLA match (antigen match vs mismatch). For OS and RFS endpoints, hazard ratios with their corresponding 95% CIs were obtained using forward selection analysis and indicated for every significant prognostic factor.

Prognostic value of CD34+/CD38- cell burden at diagnosis

Utilizing R's 'OptimalCutpoints' package [10] we identified a 6% cut-off to differentiate between patients suffering from relapse and patients staying in remission. The CD34+/CD38- cell burden at diagnosis, defined using this 6% cut-off value, also had a significant impact on RFS and OS when patients with a normal karyotype (*P*=.002 and *P*=.05 respectively, Supplementary Figures S2A, S2B) and patients diagnosed with *de novo* AML (*P*<.001 and *P*<.001 respectively, Supplementary Figures S3A and S3B) were regarded separately. Kaplan-Meier-plots utilizing a 2%, 7.5% or 10% cut-off point for the CD34+/CD38- cell burden at diagnosis for the entire cohort are shown in the supplemental Figures (for 2% cut-off: RFS *P*=.008, Supplementary Figure S4A and OS *P*=.03, Supplementary Figure S4B; for 7.5% cut-off: RFS *P*<.001, Supplementary Figure S4C and OS *P* = .01, Supplementary Figure S4D; for 10% cut-off: RFS *P*=.12 and OS *P*=.04).

CD34+/CD38- cell burden at diagnosis in patients transplanted in first CR

We performed an analysis restricted to patients transplanted in first CR (n=140; Supplementary Table SIII) excluding patients receiving HSCT in second CR. Similar to the whole cohort, we observed that patients with a high CD34+/CD38- cell burden at diagnosis were less likely to belong to the ELN Favorable Genetic Group (6% vs 32%) and more likely to belong to the ELN Adverse Genetic Group (56% vs 21%, *P*=.01) and were also more likely to harbor secondary disease (*P*=.01). While in the whole patient cohort a lower platelet count and trend for higher WBC in patients with a high CD34+/CD38- cell burden at diagnosis was detected, we did not observe significant differences in this subanalysis, which is likely a consequence of the lower patient numbers in this analysis. However, the median cell counts suggested similar trends (median platelet count 38 $x10^9$ vs 75 $x10^9$ and median WBC 18.9 $x10^9$ vs 7.2 $x10^9$) as in the whole cohort. Furthermore, a significantly lower frequency of *NPM1* mutations was observed in patients with a high CD34+/CD38- cell burden transplanted in first CR (*P*=.01). None of the patients receiving HSCT in first CR with a high CD34+/CD38- cell burden at diagnosis were *NPM1* mutated. This observation may be in line with the observation that *NPM1* mutations commonly appear in AML with the more mature CD34- phenotypes [11].

Similar to the whole cohort, a high CD34+/CD38- cell burden at diagnosis associated with shorter RFS (*P*=.01, Supplementary Figure S5A) and OS (*P*=.03, Supplementary Figure S5B) in patients transplanted in first CR. When we restricted our analysis to patients receiving NMA-HSCT in first CR, patients with a high CD34+/CD38- cell burden at diagnosis (n=15) by trend had shorter RFS (*P*=.07, Supplementary Figure S5C) and OS (*P*=.12, Supplementary Figure S5D) compared to individuals with a low burden (n=84).

Landmark analysis for patients surviving longer than 100 days after HSCT

To exclude possible aGvHD interaction effects, we performed a landmark analysis for the 160 patients that survived longer than 100 days after HSCT and also observed a significantly shorter

RFS (*P*<.001, Supplementary Figure S6A) and OS (*P*=.001, Supplementary Figure S6B) for patients with a high CD34+/CD38- cell burden at diagnosis.

Prognostic impact of the CD34+/CD38- cell burden within the ELN Genetic Risk groups

To evaluate whether the CD34+/CD38-cell burden at diagnosis adds prognostic information to the current ELN Genetic Risk classification [1], we assessed the prognostic impact of the CD34+/CD38- cell burden at diagnosis within the four groups. However, because of the limited patient number with a high CD34+/CD38- cell burden within the ELN Favorable (n=1), ELN Intermediate-I (n=6) and ELN Intermediate-II Genetic Group (n=2), they were analyzed together. Within these three groups, patients with a high CD34+/CD38- cell burden at diagnosis had shorter RFS (*P*<.001, Supplementary Figure S7A) and OS (*P*=.004, Supplementary Figure S7B) while no impact of the CD34+/CD38- cell burden in patients within the ELN Adverse Genetic Group (RFS *P*=.47, OS *P*=.61) was observed. Interestingly, patients within the ELN Favorable, Intermediate-I or Intermediate-II Genetic Groups who had a high CD34+/CD38- cell burden at diagnosis showed a trend for shorter RFS (*P*=.06, Supplementary Figure S8A) and comparable OS (*P*=.11, Supplementary Figure S8B) to patients within the ELN Adverse Genetic Group. This data suggests that a high CD34+/CD38- cell burden at diagnosis identifies poor-risk patients independently of the ELN Genetic Groups.

Comparison of the CD34+/CD38- cell burden in relapsed patients vs patients in remission

To investigate whether the CD34+/CD38- cell burden at diagnosis impacts on relapse when no cut-off is used, we compared the CD34+/CD38- cell burden in patients who suffered from relapse and patients in continuous CR after HSCT. Patients that suffered from relapse had a significantly higher CD34+/CD38- cell burden at diagnosis (median 1%, range 0-60%) than patients who remained in remission after HSCT (median 0.5%, range 0-89%; *P*=.01).

CD34+ and CD34+/CD38+ cell count at diagnosis

To evaluate whether the CD34+ or CD34+/CD38+ cell population at diagnosis associates with relapse after allogeneic HSCT we compared both populations in patients who suffered from relapse and patients in continuous remission after HSCT. Patients who suffered from relapse did not show higher CD34+ (*P*=.31) or CD34+/CD38+ cell counts (*P*=.44) at diagnosis than patients who remained in remission after HSCT. Furthermore, we observed no correlation between the CD34+/CD38- cell count and the CD34+ cell count in BM at diagnosis.

The CD34+/CD38- cell population during disease course

To assess if the adverse outcomes of some AML patients might be mediated by persisting LICs in CR after HSCT, for patients with data available, we analyzed the CD34+/CD38- cell count in CR up to 28 days before HSCT (32 before MAC-HSCT and 91 before NMA-HSCT), at day +28 after HSCT for patients in continuous CR (33 after MAC-HSCT and 99 after NMA-HSCT) and at first relapse after HSCT (8 after MAC-HSCT and 29 after NMA-HSCT).

At HSCT, patients had a median CD34+/CD38- cell count of 0.1% (range 0-2%) and on day +28 after HSCT, relapse-free patients had a median CD34+/CD38- cell count of 0.2% (range 0-5.1%). The CD34+/38- cell counts in CR before HSCT (*P*=.86) and at day +28 after HSCT (*P*=.19) did not differ significantly between patients with a high or low CD34+/CD38- cell burden at diagnosis. For patients relapsing after HSCT, we found a median CD34+/CD38- cell burden of 0.5% (0-15%). None of the patients in the low CD34+/CD38- cell burden group at diagnosis had a CD34+/CD38- cell count of over 6% at relapse, while in the high CD34+/CD38- cell burden group, 3 of 14 patients (21%) also had a CD34+/CD38- cell count of over 6% at relapse (Supplementary Figure S9). Since relapsing patients had higher, lower or similar CD34+/CD38- cell counts at relapse compared to diagnosis, we observed no association between the absolute burden of CD34+/CD38- cells at diagnosis with that at relapse. However, patients with a high CD34+/CD38- cell burden at diagnosis also had a trend towards a higher CD34+/CD38- cell

burden at relapse compared with those relapsing with a low CD34+/CD38- cell burden at diagnosis (median 0.9% vs 0.3%, *P*=.06). This might suggest a more aggressive phenotype of the underlying AML in patients with a high CD34+/CD38- cell burden at diagnosis. Vergez *et al.* [12] evaluated the CD34+CD38$_{low}$/-CD123+ cell count during relapse in 14 patients and observed a similar median burden of these cells at diagnosis (4.5%) and relapse (3.4%), and similarly to our findings, Vergez *et al.* [12] also did not observe an absolute correlation of the CD34+CD38$_{low}$/-CD123+ cell count at diagnosis and relapse. Altogether, these findings suggest that indeed the CD34+/CD38- burden at diagnosis seems to affect patients' outcome and that a small amount of LICs within the CD34+/CD38- population that cannot be detected with conventional flow cytometry in CR provokes relapse.

Comparison to the CD34+/CD38- cell count of a healthy cohort

Finally, we compared the CD34+/CD38- cell burden in AML patients at diagnosis to the CD34+/CD38- bone marrow cell counts of a healthy cohort (n=20). The healthy cohort and the AML patients were evenly matched with regard to sex (*P*=.81), but the healthy cohort was significantly younger than the AML patients (*P*=.03). We observed a median CD34+/CD38- bone marrow cell count of 0.2% (range 0-0.5%) in the healthy cohort. At diagnosis, AML patients had a significantly higher CD34+/CD38- cell burden than the healthy cohort (median 0.5% vs 0.2%; *P*=.04). Interestingly, the CD34+/CD38- cell count in AML patients in CR before HSCT was lower than the CD34+/CD38- cell count in the healthy cohort (median 0.1% vs 0.2%, *P*=.05), an effect likely caused by the preceding cytoreductive therapies. In AML patients in CR at day +28 after HSCT, the CD34+/CD38- cell count was not significantly different from the healthy cohort (median 0.2% vs. 0.2%, *P*=.42). As expected, in AML patients at relapse, the CD34+/CD38- cell count was significantly higher than that in the healthy cohort (median 0.5% vs 0.2%, *P*=.03). This was seen especially in patients with a high CD34+/CD38- cell burden at diagnosis (median 0.9%

vs 0.2%; *P*=.009), but not in patients with a low CD34+/CD38- cell burden at diagnosis (median 0.3% vs 0.2%, *P*=.18).

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Supplementary Tables

Supplemental Table SI. Additional clinical characteristics of NMA-HSCT or MAC-HSCT treated AML patients according to the CD34+/CD38- cell burden, < 6% vs ≥ 6%, at diagnosis (n=169)

Characteristic	All patients (n=169)	low CD34+/CD38- cell burden (n = 144)	high CD34+/CD38- cell burden (n = 25)	*P*
Cytogenetic and molecular genetic information				
Monosomal karyotype, n (%)				.004
Absent	146 (92)	129 (95)	17 (74)	
Present	13 (8)	7 (5)	6 (26)	
Complex karyotype, n (%)				.02
Absent	144 (89)	126 (92)	18 (75)	
Present	17 (11)	11 (8)	6 (25)	
CBF-AML, n (%)				.22
Absent	146 (92)	123 (90)	23 (100)	
Present	13 (8)	13 (10)	0 (0)	
Trisomy 8, n (%)				1
Absent	144 (89)	123 (89)	21 (91)	
Present	18 (11)	16 (11)	2 (9)	
-5/del(5q), n (%)				.004
Absent	150 (92)	133 (95)	19 (74)	
Present	13 (8)	7 (5)	6 (26)	
-7/del(7q), n (%)				.52
Absent	143 (87)	123 (88)	20 (88)	
Present	21 (13)	17 (12)	4 (12)	
FLT3-TKD, n (%)				.47
Absent	124 (87)	106 (86)	18 (95)	
Present	18 (13)	17 (14)	1 (5)	
HSCT related information				
Conditioning, n (%)				.15
NMA	120 (71)	99 (69)	21 (84)	
MAC	49 (29)	45 (31)	4 (16)	
Remission at HSCT, n (%)				.15
CR1	140 (83)	122 (85)	18 (72)	
CR2	29 (17)	22 (15)	7 (28)	
HLA type, n (%)				.30
Match	131 (78)	114 (79)	17 (68)	
Mismatch	38 (22)	30 (21)	8 (32)	
Donor, n (%)				.29
Related	36 (23)	33 (23)	3 (12)	
Unrelated	133 (77)	111 (77)	22 (88)	
Sex of the donor, n (%)				1
Male	110 (66)	93 (66)	17 (68)	
Female	56 (34)	48 (34)	8 (32)	

aGvHD, n (%)				.13
Absent	104 (71)	91 (74)	13 (57)	
Present	42 (29)	32 (26)	10 (43)	
cGvHD, n (%)				.44
Absent	54 (38.7)	47 (41)	7 (50)	
Limited	20 (16.1)	17 (15)	3 (21)	
Extended	56 (45.2)	50 (45)	4 (29)	

aGvHD, acute graft-versus-host disease; BM, bone marrow; CBF, core-binding factor; cGvHD, chronic graft-versus-host disease; CR, complete remission; FLT3-TKD, tyrosine kinase domain mutation in the FLT3 gene; HSCT, hematopoietic stem cell transplantation; HLA, human leukocyte antigen; MAC, myeloablative conditioning; NMA, non-myeloablative conditioning.

Supplementary Table SII: RFS, OS, CIR and NRM in MAC-HSCT and NMA-HSCT treated patients, n=169

	RFS	OS	CIR	NRM
At 2 years after HSCT				
All patients (n=169)	57% CI 50-65%	64% CI 56-72%	30% CI 23-37%	13% CI 8-19%
MAC-HSCT (n=49)	73% CI 61-87%	83.0% CI 73-95%	23% CI 12-36%	4% CI 1-13%
NMA-HSCT (n=120)	51% CI 42-61%	55.2% CI 47-65%	32% CI 24-41%	17% CI 11-25%
At 3 years after HSCT				
All patients (n=169)	54% CI 47-62%	62% CI 55-70%	32% CI 25-40	14% CI 9-20%
MAC-HSCT (n=49)	70% CI 58-85%	83% CI 73-95%	26% CI 14-39%	4% CI 1-13%
NMA-HSCT (n=120)	47% CI 39-58%	53% CI 44-63%	35% CI 26-44%	18% CI 12-26%
At 5 years after HSCT				
All patients (n=169)	50% CI 42-59%	57% CI 49-66%	36% CI 28-44%	14% CI 9-20%
MAC-HSCT (n=49)	60% CI 45-80%	75% CI 63-91%	36% CI 19-53%	4% CI 1-13%
NMA-HSCT (n=120)	46% CI 37-56%	49% 40-60%	36% CI 27-46%	18% CI 12-26%

CI, confidence interval; CIR, cumulative incidence of relapse; MAC, myeloablative conditioning; NMA, non-myeloablative conditioning, NRM, non-relapse mortality; OS, overall survival; RFS, relapse-free survival.

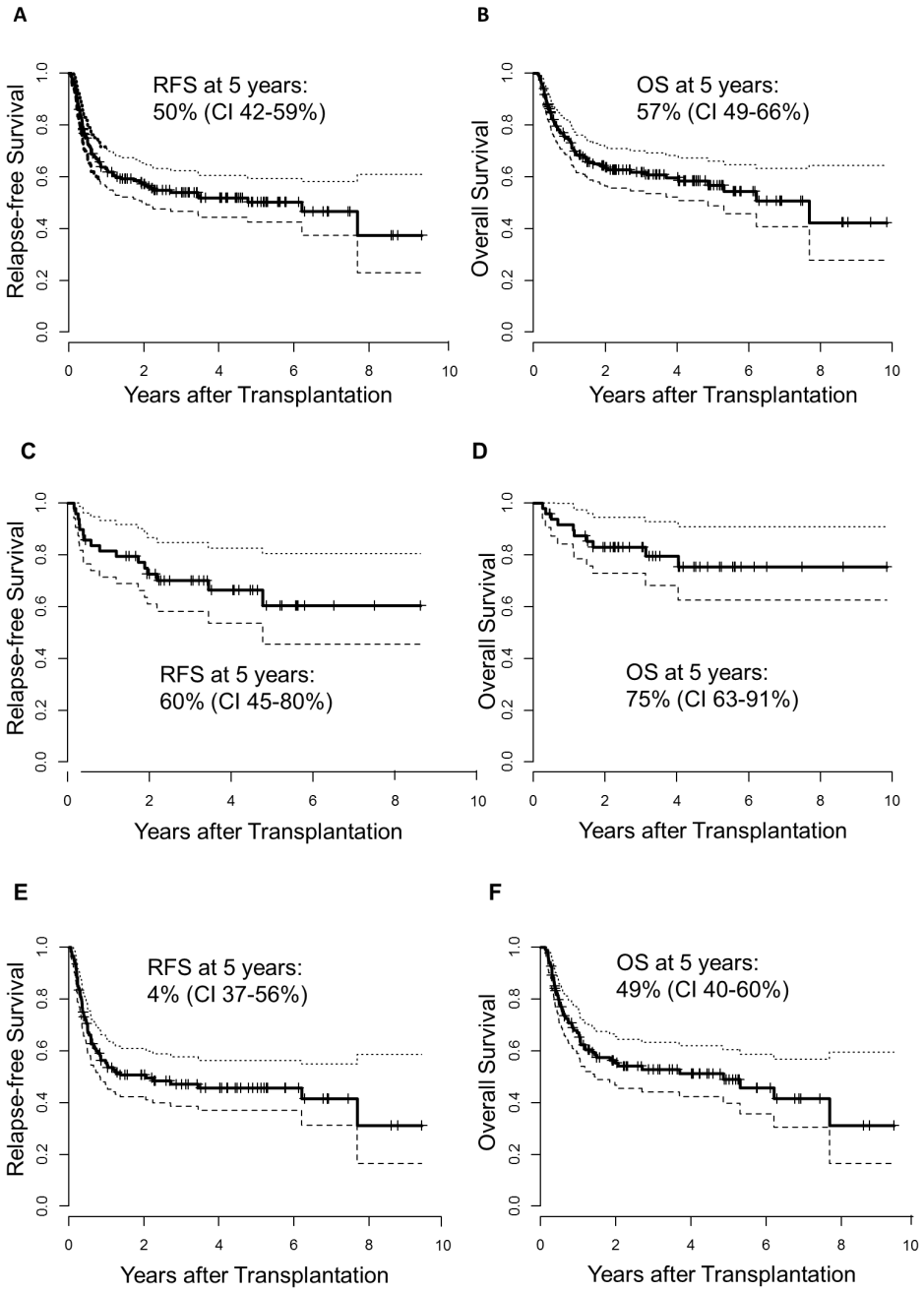
Supplementary Table SIII: Subgroup analysis of associations according to the CD34+/CD38- cell burden, < 6% vs ≥ 6%, at diagnosis for patients receiving NMA-HSCT or MAC-HSCT in CR1 (n=140)

Characteristic	All CR1 patients (n=140)	low CD34+/CD38- cell burden (n = 122)	high CD34+/CD38- cell burden (n = 18)	*P*
Age at HSCT, years				.19
Median	61.5	61	62	
Range	19.4-75.3	19-75	30-74	
Sex, n (%)				.62
Male	68 (49)	58 (48)	10 (56)	
Female	72 (51)	64 (52)	8 (44)	
Hemoglobin, g/dL				.83
Median	8.5	8.5	9.1	
Range	4.5-15.7	4.5-15.7	5.3-13.2	
Platelet count, x 10^9/L				.18
Median	64	75	38	
Range	2-327	2-327	13-178	
WBC count, x 10^9/L				.27
Median	7.2	7.2	18.9	
Range	0.7-385	0.7-385	1.1-295	
Percentage of blood blasts, %				.42
Median	24	22	39	
Range	0-98	0-98	2-97	
Percentage of BM blasts, %				.43
Median	56.5	57	52	
Range	0-95	0-95	0-95	
Karyotype, n (%)				.32
Abnormal	73 (54)	61 (53)	12 (67)	
Normal	61 (46)	55 (47)	6 (33)	
ELN Genetic Group, n (%)				.01
Favorable	36 (29)	35 (32)	1 (6)	
Intermediate-I	28 (22)	24 (22)	4 (25)	
Intermediate-II	29 (23)	27 (25)	2 (13)	
Adverse	32 (26)	23 (21)	9 (56)	
Disease origin, n (%)				.01
De novo	93 (66)	86 (70)	7 (39)	
Secondary	47 (34)	36 (30)	11 (61)	
MDS	29	23	6	
MPN	9	5	4	
Solid tumor	9	8	1	
NPM1, n (%)				.01
Wild-type	93 (76)	77 (73)	16 (100)	
Mutated	29 (24)	29 (27)	0 (0)	
FLT3-ITD, n (%)				1
Absent	99 (83)	87 (82)	12 (86)	
Present	21 (17)	19 (18)	2 (14)	
CEBPA, n (%)				.21
Wild-type	94 (86)	83 (83)	11 (100)	
Mutated	17 (13)	17 (17)	0 (0)	

BM, bone marrow; ELN, European LeukemiaNet; FLT3-ITD, internal tandem duplication of the FLT3 gene; HSCT, hematopoietic stem cell transplantation; MAC, myeloablative; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasia; NMA, non-myeloablative; WBC, white blood cell.

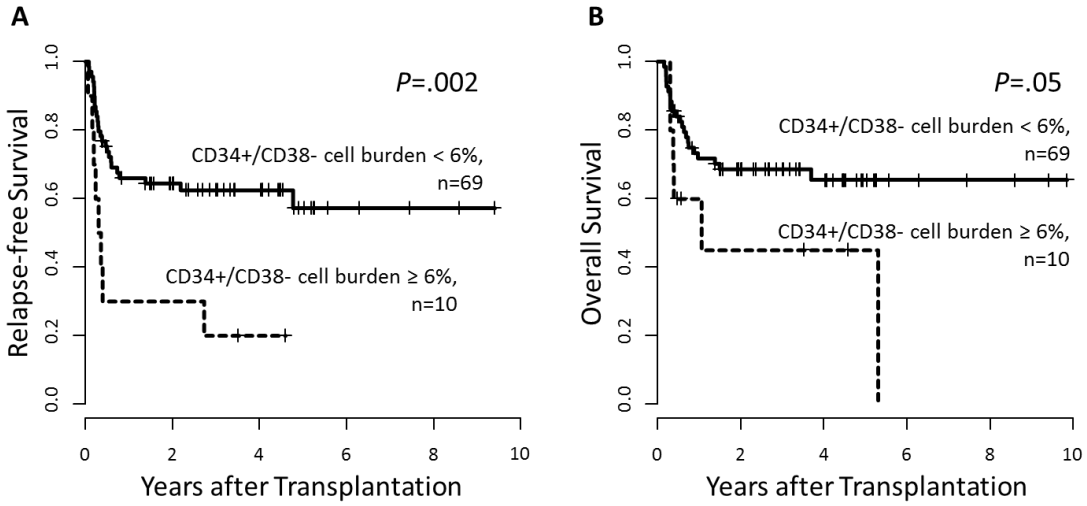
Supplementary Figures

Supplementary Figure S1



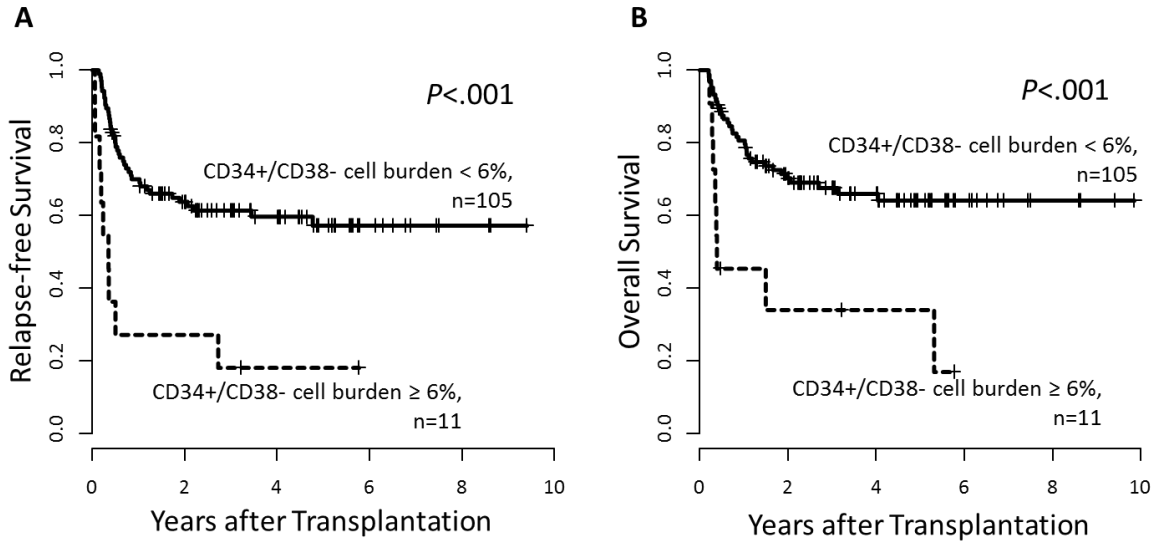
Supplementary Figure S1. Outcome for the whole cohort. Relapse-free survival (A) and overall survival (B) in NMA- or MAC-HSCT-treated AML patients, n=169; relapse-free survival (C) and overall survival (D) in MAC-HSCT-treated AML patients, n=49; relapse-free survival (E) and overall survival (F) in NMA-HSCT-treated AML patients, n=120.

Supplementary Figure S2



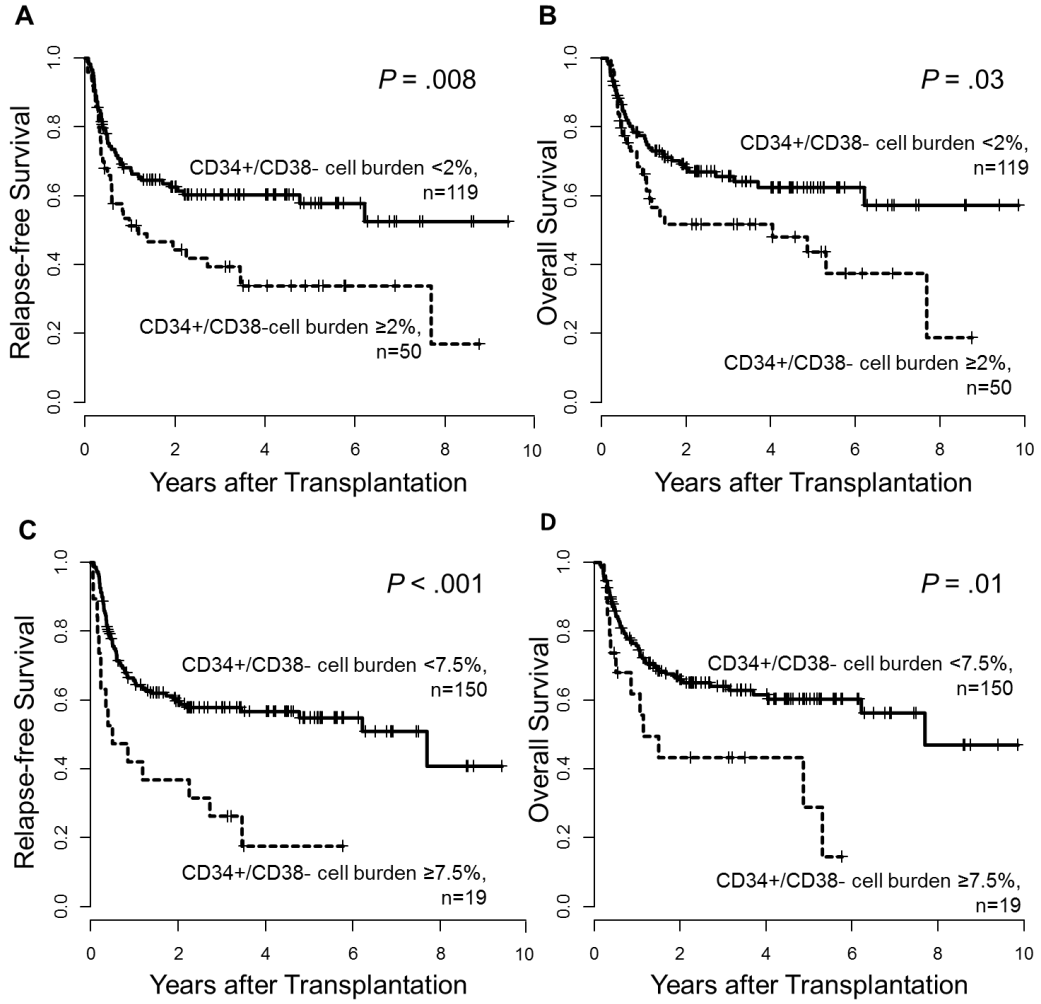
Supplementary Figure S2. Survival in NMA- or MAC-HSCT-treated patients with normal karyotype AML according to CD34+/CD38- cell burden at diagnosis, < 6% vs ≥ 6%, n=79. (A) Relapse-free Survival. (B) Overall Survival.

Supplementary Figure S3



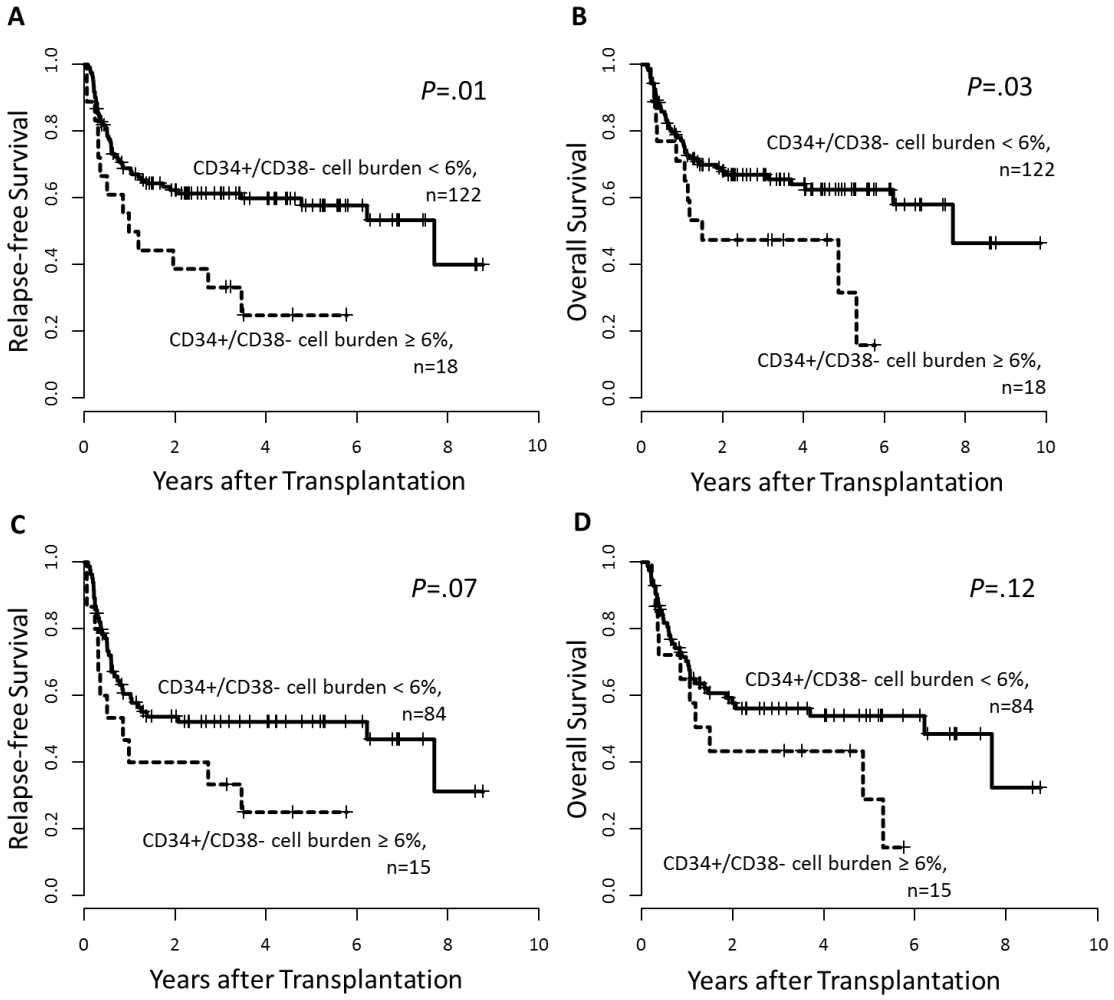
Supplementary Figure S3. Survival in NMA- or MAC-HSCT-treated patients with *de novo* AML according to CD34+/CD38- cell burden at diagnosis, < 6% vs ≥ 6%, n=116.
(A) Relapse-free Survival. (B) Overall Survival

Supplementary Figure S4



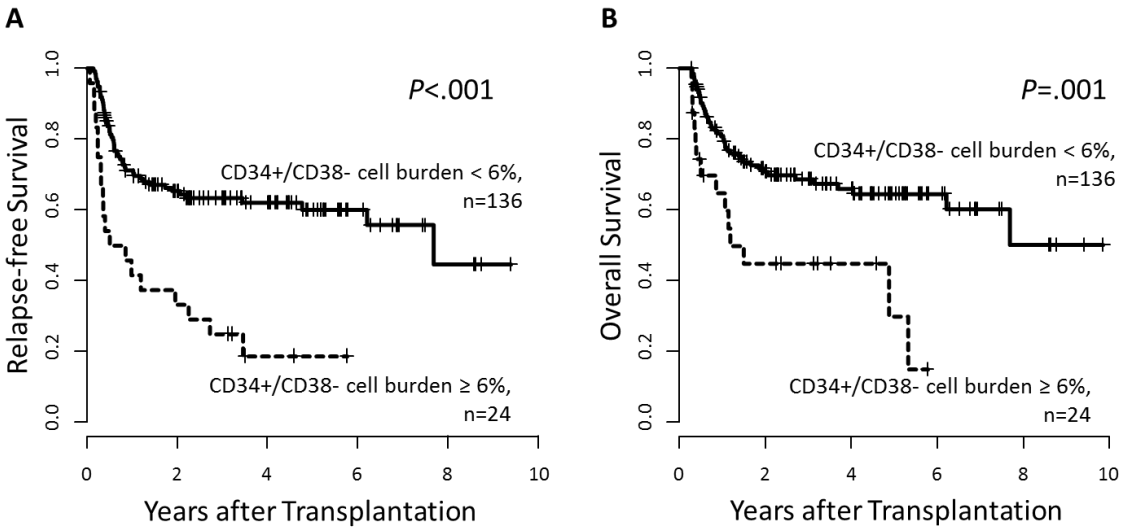
Supplementary Figure S4. Survival according to alternative cut-offs. Relapse-free Survival (A) and Overall Survival (B) in NMA-HSCT or MAC-HSCT-treated patients according to CD34+/CD38- cell burden at diagnosis, <2% vs ≥ 2%, n=169. Relapse-free Survival (C) and Overall Survival (D) in NMA- or MAC-HSCT-treated patients according to CD34+/CD38- cell burden at diagnosis, <7.5% vs ≥ 7.5%, n=169.

Supplementary Figure S5



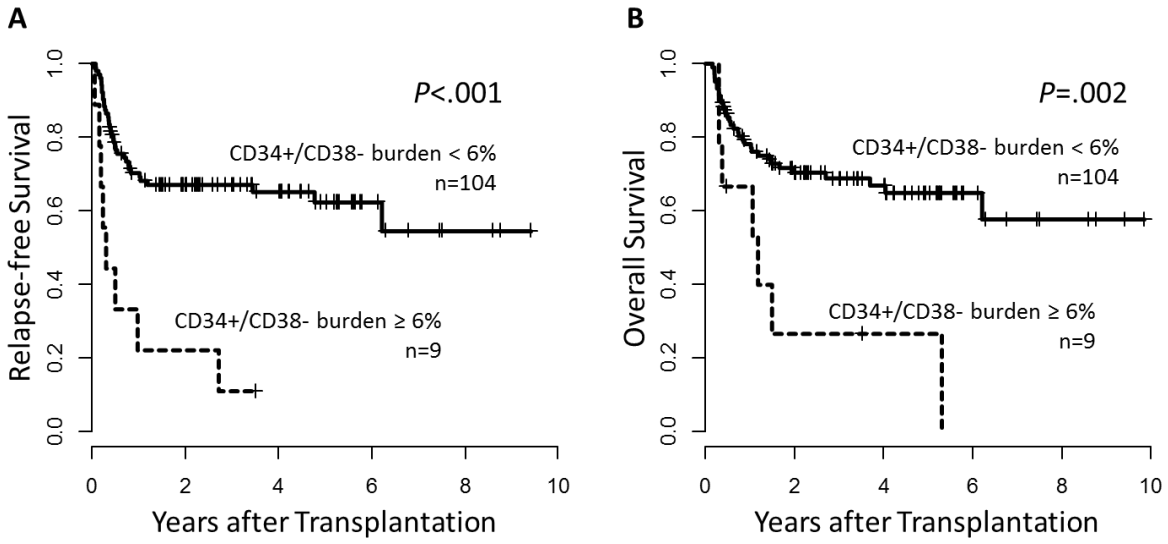
Supplementary Figure S5. Subanalysis for patients transplanted in CR1. Relapse-free Survival (A) and Overall Survival (B) in all patients receiving HSCT in CR1 (n=140) and Relapse-free Survival (C) and Overall Survival (D) in patients receiving NMA-HSCT in CR1 (n=99) according to CD34+/CD38- cell burden at diagnosis, < 6% vs ≥ 6 %.

Supplementary Figure S6



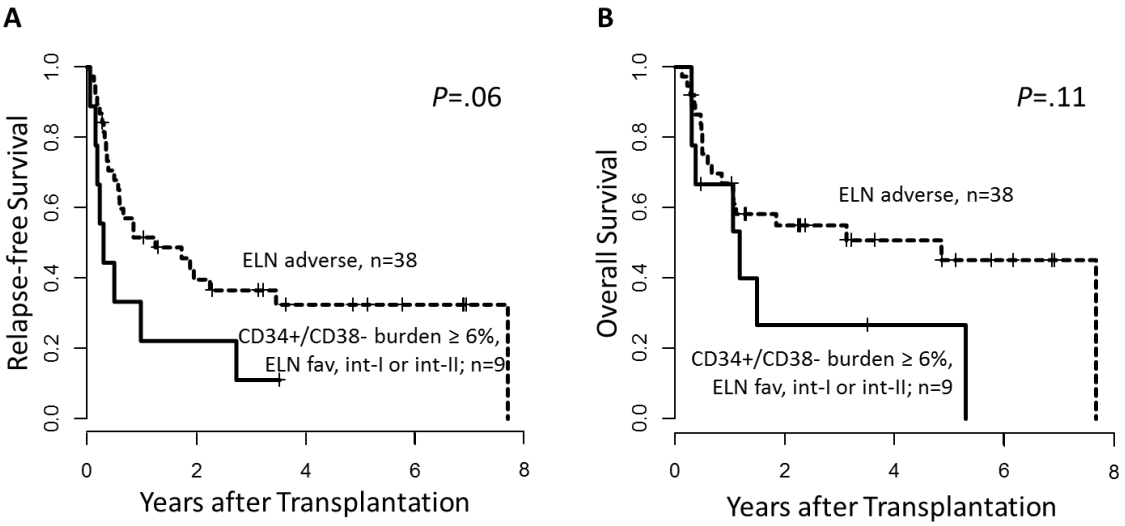
Supplementary Figure S6. Landmark analysis for patients surviving longer than 100 days after HSCT. Relapse-free Survival (A) and Overall Survival (B) in NMA- or MAC-HSCT-treated patients according to CD34+/CD38- cell burden at diagnosis, < 6% vs ≥ 6%, n=160.

Supplementary Figure S7



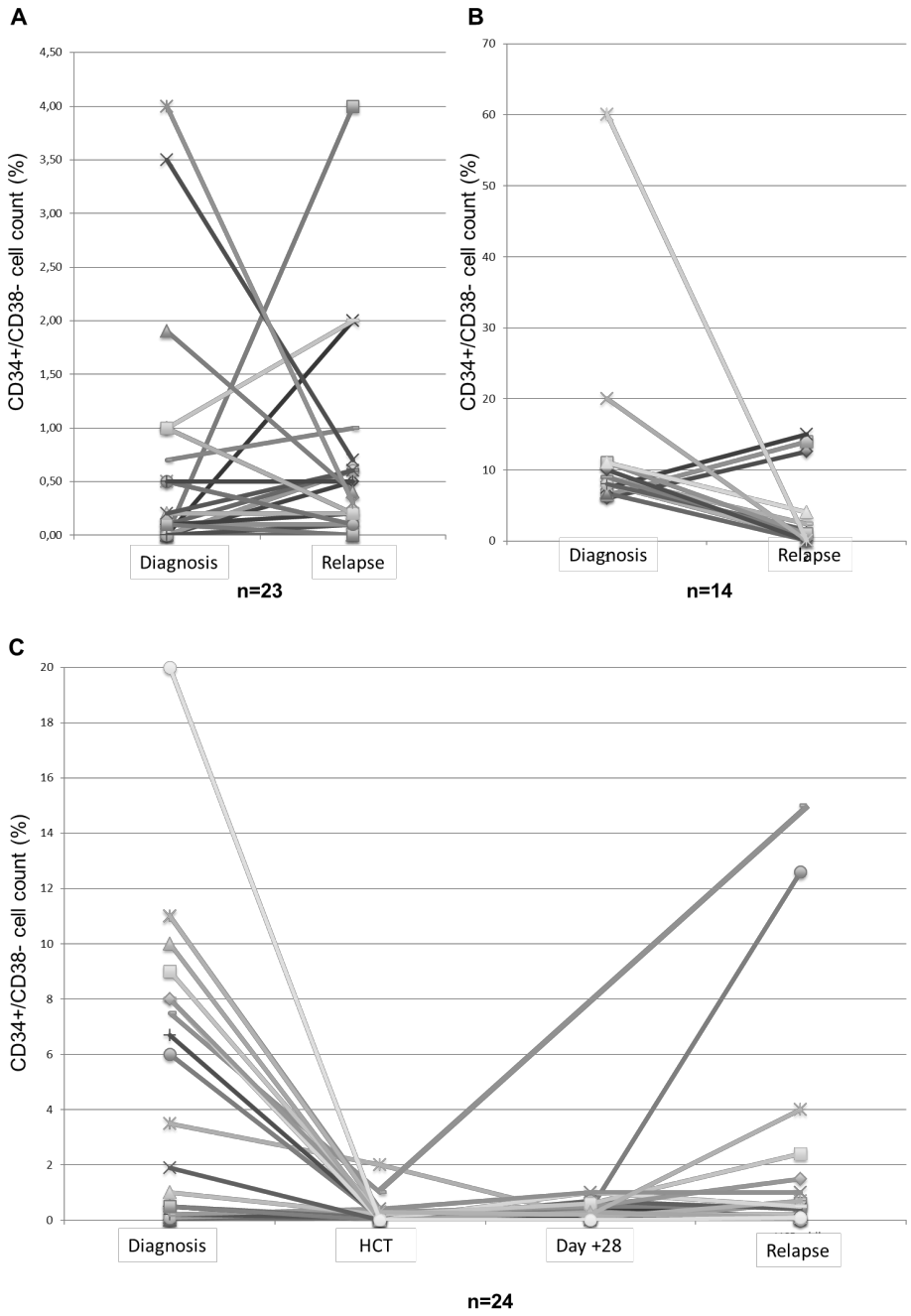
Supplementary Figure S7. Subanalysis for patients with ELN favorable, intermediate-I and intermediate-II Genetic Risk. Relapse-free Survival (A) and Overall Survival (B) in in NMA- or MAC-HSCT-treated patients according to CD34+/CD38- cell burden at diagnosis, < 6% vs ≥ 6 %, n=113.

Supplementary Figure S8



Supplementary Figure S8. Comparison between patients with ELN adverse Genetic Risk (n=38) and patients with ELN favorable, intermediate-I or intermediate-II Genetic Risk and a high CD34+/CD38- cell burden at diagnosis (n=9), < 6% vs ≥ 6%. (A) Relapse-free Survival. (B) Overall Survival.

Supplementary Figure S9



Supplementary Figure S9. CD34+/CD38- cell count at diagnosis and during relapse for patients with a low (A) or a high (B) CD34+/CD38- cell burden at diagnosis. (C) CD34+/CD38- cell count during disease progression for patients with available flow data at time points.

Zusammenfassung / Summary

Dissertation zur Erlangung des akademischen Grades Dr. med.

Prognostic Impact of the CD34+/CD38- Cell Burden in Patients with Acute Myeloid Leukemia receiving Allogeneic Stem Cell Transplantation

eingereicht von Barbara Madlen Jentzsch

angefertigt am

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Department für Innere Medizin, Neurologie und Dermatologie

Selbstständige Abteilung für Hämatologie und Internistische Onkologie

betreut von Prof. Dr. med. Dr. h.c. Dietger Niederwieser

Mai 2017

Although over the last decades important steps towards understanding the pathogenesis of acute myeloid leukemia (AML) have been made, outcomes for most AML patients remain vastly improvable. On our way to individualized therapies, risk assessment at diagnosis of AML is crucial to estimate individual treatment response and probability of relapse.

The identification of new prognostic factors can lead to optimized risk stratification, adjustment of treatment strategies as well as to the detection of new potential therapeutic targets and subsequently may improve prognosis for AML patients. Leukemia initiating cells

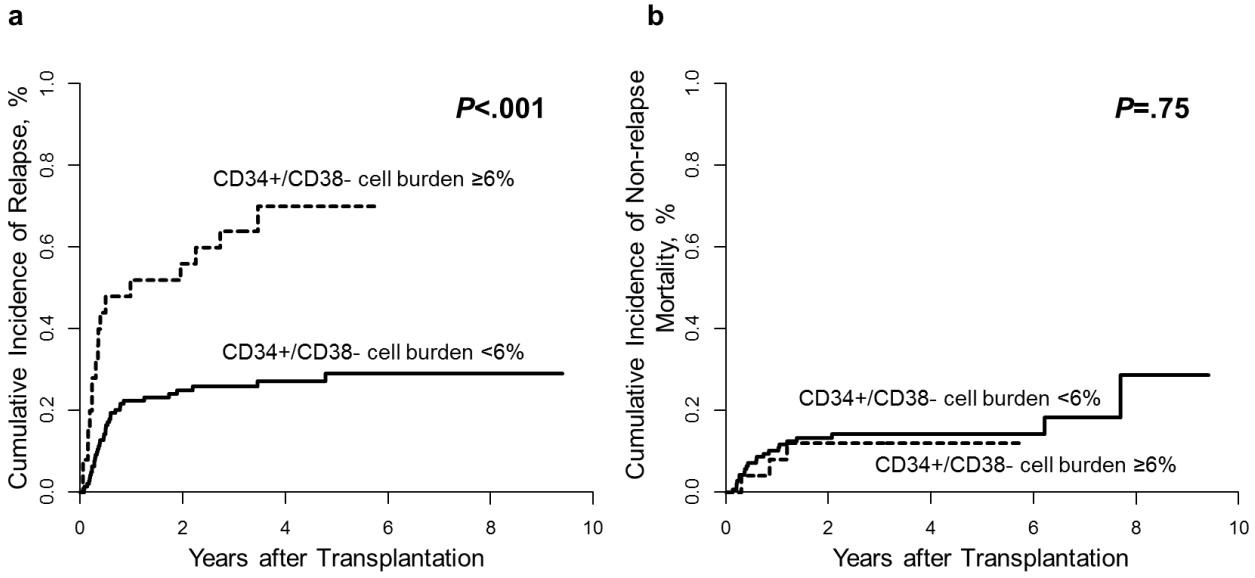
(LICs) are an immature cell population that is thought to be responsible for the development, maintenance, and recurrence of AML. Much effort has been made to describe the phenotype of these cells and most researchers agree that LICs express CD34 in the absence of CD38. In some studies a high burden of LICs at diagnosis impacted adversely on outcomes when AML patients were consolidated with chemotherapy. However, the prognostic impact of a high LIC burden in AML patients receiving allogeneic hematopoietic stem cell transplantation (HSCT) as consolidation therapy – where the therapeutic effect also relies on graft-versus-leukemia effects - remained unknown.

The first objective of the here presented study was to analyze the prognostic impact of the bone marrow CD34+/CD38- LIC population at diagnosis in AML patients who received an allogeneic HSCT for consolidation therapy.

Analyzing the CD34+/CD38- cell burden at diagnosis in our HSCT-treated cohort, we observed this population to be highly variable, ranging from 0% to 89% of bone marrow cells with a median of 0.5% at diagnosis. AML patients with a high CD34+/CD38- cell burden (over 6%) at diagnosis had a significantly shorter overall survival (P=.005) and relapse-free survival (P<.001). This was also observed when we restricted our analysis to patients with normal karyotype, *de novo* disease or HSCT in first complete remission.

The observed difference in survival was mediated by a higher cumulative incidence of relapse in patients with a higher CD34+/CD38- cell burden at diagnosis (P<.001, **Figure 1a**). In contrast, the cumulative incidence of non-relapse mortality which summarizes HSCT-related deaths due to graft-versus-host disease or infections, as well as mortality not associated with HSCT or relapse was similar in both groups (P=.75, **Figure 1b**).

<u>Figure 1:</u> Cumulative Incidence of relapse (a) and Cumulative Incidence of Non-relapse Mortality (b) in 169 AML patients receiving HSCT depending on the CD34+/CD38- burden at diagnosis (<6% *vs.* ≥6%)



The second objective of the here presented study was <u>to uncover possible associations of the bone marrow CD34+/CD38- LIC population at diagnosis with other clinical and molecular characteristics and prognostic factors in AML</u>.

The patients in our set were well characterized for other clinical and genetic factors described in AML. We observed that patients with a higher CD34+/CD38- burden at diagnosis had a lower platelet count at diagnosis (*P*=.04), were also more likely to belong to worse ELN 2010 genetic groups (*P*=.001) and more often had complex (*P*=.02) or monosomal karyotypes (*P*=.004), for which a dismal prognosis have been described. Furthermore, AML with secondary origin following MDS or MPN diagnosis is known to associate with a worse prognosis than *de novo* disease. We also observed a higher CD34+/CD38- cell burden in patients with secondary AML (*P*=.009) compared to *de novo* disease. On the other hand, *CEBPA* mutations, which frequently occur in AML with favorable outcomes were not observed in patients with high CD34+/CD38- cell burden (*P*=.13).

The third objective of our study was to evaluate whether the bone marrow CD34+/CD38- LIC population at diagnosis refines todays risk stratification in AML.

As described above, a high CD34+/CD38- cell burden associated with other known adverse prognosticators in AML. Strikingly, a multivariable analysis demonstrated that the CD34+/CD38- cell burden at diagnosis impacted on relapse-free and overall survival, independently of other prognostic factors. The risk of relapse or death in patients with a high CD34+/CD38- cell burden at diagnosis was about twice as high as in patients with a low CD34+/CD38- cell burden at diagnosis. This data suggests that the immature CD34+/CD38- cell population may contribute to the dismal outcome of patients with adverse karyotypes or secondary AML. Finally, we were able to show that patients with favorable or intermediate risk according to the ELN 2010 genetic risk classification but a high CD34+/CD38- cell burden at diagnosis had a trend for shorter relapse-free survival (P=.06) and comparable overall survival (P=.11) as patients within the Adverse ELN 2010 Genetic Group. Thus, a high CD34+/CD38- cell burden may be able to identify poor-risk patients also independently of the ELN 2010 Genetic Groups.

In conclusion, we were the first to show that a high burden of a LIC-containing cell population at diagnosis also associates with worse outcomes in AML patients who received an allogeneic HSCT for consolidation therapy. Strikingly, this observation was independent of other known prognostic factors in AML. Our work further improves risk assessment at AML diagnosis and points out the need to develop therapies against the clinically important LIC population. In the future, LIC-targeted therapies may overcome the adverse impact of a high CD34+/CD38- cell burden and improve survival of AML patients.

Weiterführende Arbeiten / Future developments

GPR56 as new LIC marker

Although the here presented CD34+/CD38- cell phenotype remains the widely accepted LIC population, there is ongoing research for new stem cell markers to more precisely define the LIC population. With the introduction of experiments using more permissive immunodeficient mouse models (e.g. NOD/SCID/IL2r-/-, NOD/SCID/2m-/-), it was suggested that, in some cases, the CD34- cell population might also harbor a small amount of cells with LIC potential, e.g. in *NPM1* mutated AML.[74]

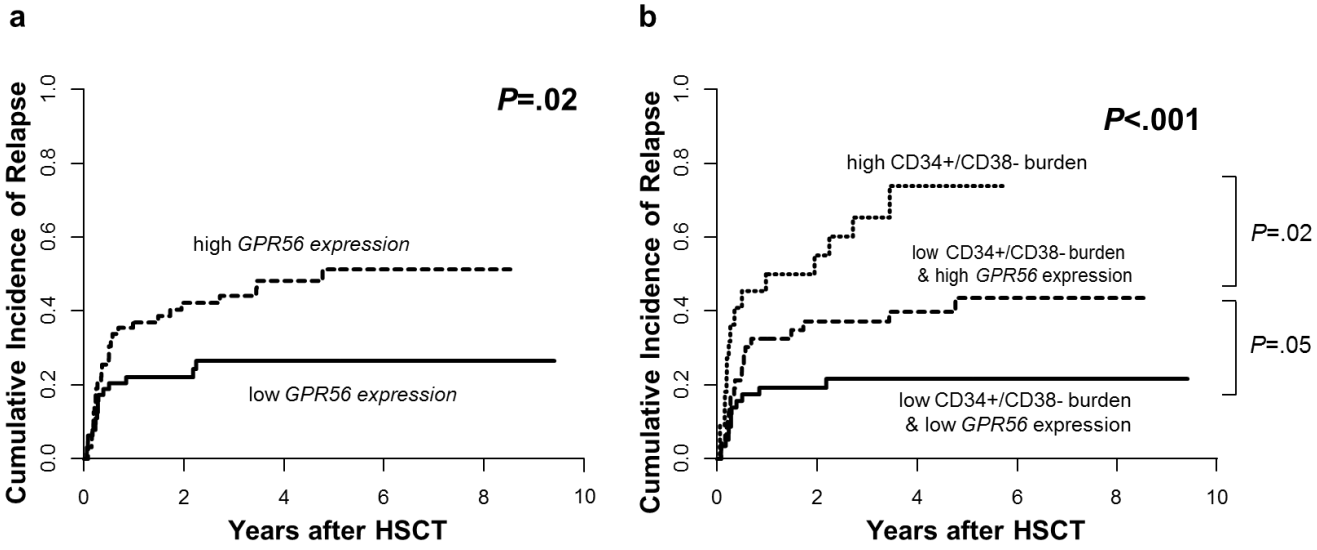
Recently, the G protein-coupled receptor 56 (GPR56) was described as a new LIC marker.[75] GPR56 is a G protein-coupled adhesion molecule regulating survival, migration and adhesion in various cell types.[76] In AML, GPR56 seems to be important for the interaction of LICs with the bone marrow stroma, called stem cell niche.[75] *GPR56* expression was shown to be upregulated in healthy hematopoietic stem cells as well as LICs, especially when residing in a quiescent state, and downregulated during maturation.[77] High GPR56 expressing AML cells were able to serially transplant leukemia in NOD/SCID mice and thus, were functionally validated as LICs.[78,79] Interestingly, this property was also found in LICs with low or absent CD34 expression, suggesting GPR56 as a new marker with the ability to define LICs independently of the CD34 expression.[78]

In our here presented study, most patients with a CD34+/CD38- cell burden of 6% or higher at diagnosis (15% of the used set) had a dismal outcome. However, within the larger group of patients with a lower CD34+/CD38- cell burden (85% of the used set) outcome remained highly heterogeneous. Since GPR56 may provide further information on the LIC containing AML cell population, we evaluated the diagnostic *GPR56* expression at diagnosis in 148 AML patients with adequate material available in a follow up work.[80] Here, we observed a correlation of the *GPR56* expression with the CD34+/CD38- cell burden (*P*=.002) at

diagnosis. As expected, the *GPR56* expression at diagnosis was associated with a higher cumulative incidence of relapse after HSCT (*P*=.02, **Figure 2a**).

Since GPR56 was described to define LICs also in the CD34- compartment, we further evaluated the *GPR56* expression in the subgroup of patients with a low CD34+/CD38- cell burden at diagnosis. Interestingly, a high *GPR56* expression defined a patient population with significantly higher cumulative incidence of relapse compared to patients with a low *GPR56* expression and a low CD34+/CD38- cell burden (*P*=.05) but significantly lower cumulative incidence of relapse compared to patients with a high CD34+/CD38- cell burden at diagnosis (*P*=.02, **Figure 2b**).[80]

Figure 2: Cumulative incidence of relapse according to (a) the *GPR56* expression status at diagnosis (high *vs.* low, median cut) and (b) the *GPR56* expression status (high *vs.* low, median cut) and the CD34+/CD38- cell burden at diagnosis (high *vs.* low, 6% cut)



This observation was confirmed in a multivariable analysis, in which the combined information on the LIC burden at diagnosis, defined by the CD34+/CD38- cell burden and the

GPR56 expression, significantly impacted on cumulative incidence of relapse after adjustment for disease status at HSCT and *EVI1* expression at diagnosis. (**Table 4**).

Table 4: Multivariable Analysis for CIR in HSCT treated AML patients (n=148)

Variable	Cumulative incidence of Relapse		
	HR*	**95% CI**	***P***
Leukemia initiating cell population (low CD34+/CD38- & low *GPR56 vs*. low CD34+/CD38- & high *GPR56 vs*. high CD34+/CD38-)	1.42	1.03-1.98	.035
Disease status at HSCT (CRi *vs*. CR)	0.43	0.22-0.85	.016
EVI1 expression at diagnosis (negative *vs*. positive)	2.58	1.29-5.15	.007

Abbreviations: CI, confidence interval; CR, complete remission; CRi, complete remission with incomplete peripheral recovery; HSCT, hematopoietic cell transplantation; HR, hazard ratio.

* HR, Hazard ratio, <1 (>1) indicate lower (higher) risk for an event for the first category listed for the dichotomous variables and for the higher values of the continuous variables. Variables considered in the models were those significant at α=0.20 in univariable analyses. Variables considered were: disease origin (secondary *vs. de novo*), ELN 2010 classification, platelet count at diagnosis, blast count in bone marrow at diagnosis, leukemia initiating cell population at diagnosis (low CD34+/CD38- & low *GPR56 vs.* low CD34+/CD38- & high *GPR56 vs.* high CD34+/CD38-), *EVI1* expression at diagnosis (negative *vs.* positive), age at HSCT, disease status at HSCT (CRi *vs.* CR).

Our data supports the assumption by Pabst *et al.* that GPR56 may further subdivide the CD34+/CD38- LIC compartment and may be an independent LIC marker. In the near future, we intend to perform *in vitro* and *in vivo* experiments with both cell populations to further explore the prognostic value and biological features of this LIC containing AML cell population.

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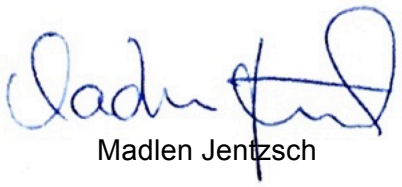

Leipzig, 25.04.2017 Madlen Jentzsch

Curriculum Vitae

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2007	1. Abschnitt der Ärztlichen Prüfung
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2009	Famulatur in der Gefäßchirurgie, Helios Klinik Borna
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2011	3. Tertial des Praktischen Jahres, Neurologie, Städtisches Klinikum Chemnitz
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Berufliche Weiterbildung

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09/2013	Grundkurs im Strahlenschutz zum Erwerb der Fachkunde für Ärzte, ermächtigte Ärzte, strahlenschutzbeauftragte für den physikalisch-technischen Bereich und Medizinphysik-Experten
09/2015	Spezialkurs im Strahlenschutz bei der Untersuchung mit Röntgenstrahlen (Diagnostik) zum Erwerb der Fachkunde für Ärzte
09/2015	GCP-Schulung für Prüfärzte und Studienteams
05/2016	Grundkurs der Versuchstierkunde mit Schwerpunkt Maus und Ratte

Mitgliedschaften in Gesellschaften

Seit 2013	Deutsche Gesellschaft für Hämatologie und Onkologie
Seit 2013	European Hematology Association
Seit 2014	Deutsche Gesellschaft für Innere Medizin

Auszeichnungen

2014	Travel Grant des 19th Annual Meeting 2014 of The European Hematology Association (EHA)
2014	Travel Grant der 120. Jahrestagung der Deutschen Gesellschaft für Innere Medizin (DGIM)
2015	ASH Abstract Achievement Award der 57. Jahrestagung der American Society of Hematology
2016	Travel Grant des 21th Annual Meeting 2016 of The European Hematology Association (EHA)
2016	ASH Abstract Achievement Award der 58. Jahrestagung der American Society of Hematology

Konferenz-Vorträge

2013	*Eine niedrige BAALC Expression ist mit längerem Überleben von Patienten mit akuter myeloischer Leukämie nach allogener Stammzelltransplantation nach Konditionierung mit reduzierter Intensität assoziiert* Jahrestagung der Deutschen Gesellschaft für Hämatologie und Onkologie (DGHO)
2014	*Ein hoher CD34+/CD38- Anteil bei Diagnose einer Akuten Myeloischen Leukämie assoziiert mit einer schlechten Prognose nach allogener Stammzelltransplantation mit reduzierter Konditionierung* Jahrestagung der Deutschen Gesellschaft für Hämatologie und Onkologie (DGHO)
2015	*Der prognostische Einfluss der MN1 Expression bei Patienten mit Akuter*

Myeloischer Leukämie (AML), die eine allogene Stammzelltransplantation in Komplettremission nach nicht-myeloablativer Chemotherapie (NMA-SCT) erhalten
Jahrestagung der Deutschen Gesellschaft für Hämatologie und Onkologie (DGHO)

2016 *High Expression of the Stem Cell Marker GPR56 is Associated with an Increased Relapse Incidence In AML after Allogeneic Stem Cell Transplantation*
Annual Meeting 2016 of The European Hematology Association (EHA)

2016 *Die Profile der Oberflächenantigene in Patienten mit Akuter Myeloischer Leukämie definieren Subgruppen mit unterschiedlicher biologischer und klinischer Charakterisierung*
Jahrestagung der Deutschen Gesellschaft für Hämatologie und Onkologie (DGHO)

2016 *Eine hohe Expression des Stammzellmarkers GPR56 ist mit einem erhöhten Rezidivrisiko in Patienten mit Akuter Myeloischer Leukämie assoziiert, die eine allogene Stammzelltransplantation erhalten*
Jahrestagung der Deutschen Gesellschaft für Hämatologie und Onkologie (DGHO)

2016 *High Blood BAALC Copy Numbers determined by ddPCR at Timepoint of Allogeneic Transplantation in Complete Remission Predicts Relapse in Patients with Acute Myeloid Leukemia.*
Jahrestagung der American Society of Hematology (ASH)

Komplette Publikationsliste

Peer-Reviewed Publications

- Wieland I, Kentouche K, **Jentzsch M**, Lothschütz D, Graf N, Sykora KW. Long-term remission of recurrent thrombotic thrombocytopenic purpura (TTP) after rituximab in children and young adults. *Pediatr Blood Cancer*. 2015; 62(5):823-9.

- Pönisch W, Plötze M, Holzvogt B, Andrea M, Schliwa T, Zehrfeld T, Hammerschmidt D, Schwarz M, Edelmann T, Becker C, Hoffmann FA, Schwarzer A, Kreibich U, Gutsche K, Reifenrath K, Schwarzbach H, Heyn S, Franke GN, **Jentzsch M**, Leiblein S, Schwind S, Lange T, Vucinic V, Al-Ali HK, Niederwieser D. Stem cell mobilization and autologous stem cell transplantation after pretreatment with bendamustine, prednisone and bortezomib (BPV) in newly diagnosed multiple myeloma. *J Cancer Res Clin Oncol*. 2015; 141(11):2013-22

- Pfrepper C, Klink A, Behre G, Schenk T, Franke G-N, **Jentzsch M**, Schwind S, Al-Ali HK, Hochhaus A, Niederwieser D, Sayer HG: Risk factors for outcome in refractory acute myeloid leukemia patients treated with a combination of fludarabine, cytarabine, and amsacrine followed by a reduced-intensity conditioning and allogeneic stem cell transplantation. *J Cancer Res Clin Oncol*. 2016;142(1):317-24

- **Jentzsch M**, Bill M, Nicolet D, Leiblein S, Schubert K, Pleß M, Bergmann U, Wildenberger K, Schuhmann L, Cross M, Pönisch W, Franke GN, Vucinic V, Lange T, BehreG, Mrózek K, Bloomfield CD, Niederwieser D, Schwind S. Prognostic Impact of the CD34+/CD38- Cell Burden in Patients with Acute Myeloid Leukemia receiving Allogeneic Stem Cell Transplantation. *Am J Hematol*. 2017; 92(4): 388-396.

- Bill M, **Jentzsch M**, Grimm J, Schubert K, Lange T, Cross M, Behre G, Vucinic V, Pönisch W, Franke GN, Niederwieser D, Schwind S. Prognostic impact of the European LeukemiaNet standardized reporting system in older AML patients receiving stem cell transplantation after non-myeloablative conditioning. *Bone Marrow Transplant*. 2017; doi: 10.1038/bmt.2017.42. [Epub ahead of print].

- Mrachacz H, Khoder N, Holzvogt M, Holzvogt B, Andrea M, Heyn S; Schliwa T, Bill M, Becker C, Schwarz M; Zehrfeld T, Pfrepper C, Franke GN, Krahl R, **Jentzsch M**, Leiblein S, Schwind S, Vucinic V, Lange T, Niederwieser D, Pönisch W. Successeful Treatment of Patients with newly diagnosed/untreated light chain Multiple Myeloma with a Combination of Bendamustine, Prednisone and Bortezomib (BPV). *Submitted to Ann Hematol*.

- **Jentzsch M**, Bill M, Nicolet D, Schuhmann L, Schubert K, Grimm J, Schulz J, Franke GN, Behre G, Pönisch W, Vucinic V, Müller-Tidow C, Pabst C, Mrózek K, Bloomfield CD, Niederwieser D, Schwind S. High expression of the new stem cell marker GPR56 defines acute myeloid leukemia patients at higher relapse risk after allogeneic stem cell transplantation independently of the CD34+/CD38- population. *In preparation*.

- **Jentzsch M**, Bill M, Schulz J, Grimm J, Schubert K, Beinicke S, Häntschel J, Pönisch W, Franke G-N, Vucinic V, Behre G, Lange T, Niederwieser D, Schwind S. High Blood *BAALC* Copy Numbers at Allogeneic Transplantation Predict Early Relapse in Patients with Acute Myeloid Leukemia. *In preparation*.

Conference Proceedings and Abstracts

- **Jentzsch M**, Lange T, Krahl R, Franke G-N, Schakols K, Cross M, Al-Ali HK, Vucinic V, Niederwieser D, Schwind S: *Prognostic significance of aberrant expression of BAALC, ERG and MN1 in patients with acute myeloid leukemia undergoing allogenic hematopoietic cell transplantation with reduced-intensity conditioning,* Presented at The annual meeting 2013 of the European Society of Blood and Bone Marrow Transplantation (EBMT) (Abstract #P1102)

- Schwind S, **Jentzsch M**, Lange T, Pönisch W, Heyn S, Vucinic V, Franke G-N, Krahl R, Jäkel N, Al-Ali HK, Cross M, Behre G, Marcucci G, Bloomfield CD, Niederwieser D: *Low Pre-Treatment mir-181a-1 and mir-181a-2 Expression associate with Relapse in Intermediate Risk Acute Myeloid Leukemia after Reduced-Intensity Conditioning Allogeneic Transplantation,* Presented at the Annual Meeting 2013 of The European Hematology Association (EHA) (Abstract #P362)

- **Jentzsch M**, Lange T, Krahl R, Franke G-N, Schakols K, Cross M, Al-Ali HK, Vucinic V, Niederwieser D, Schwind S: *Eine niedrige BAALC Expression ist mit längerem Überleben von Patienten mit akuter myeloischer Leukämie nach allogener Stammzelltransplantation nach Konditionierung mit reduzierter Intensität assoziiert,* Presented at The Annual Meeting 2013 ot the Deutsche Gesellschaft für Hämatologie und Onkologie (DGHO) (Abstract #V444)

- Schwind S, **Jentzsch M**, Lange T, Pönisch W, Heyn S, Vucinic V, Franke G-N, Krahl R, Jäkel N, Al-Ali HK, Cross M, Behre G, Marcucci G, Bloomfield CD, Niederwieser D: *Höhere vortherapeutische miR-181a-1 und/oder miR-181a-2 Expression in Patienten mit intermediär-Risiko akuter myeloischer Leukämie ist mit einer niedrigeren Rezidivrate nach allogener hämatopoietischer Stammzelltransplantation nach Konditionierung mit reduzierter Intensität,* Presented at The Annual Meeting 2013 ot the Deutsche Gesellschaft für Hämatologie und Onkologie (DGHO) (Abstract #V652)

- Bill M, **Jentzsch M**, Lange T, Kloss L, Krahl R, Franke G-N, Fricke S, Vucinic V, Pönisch W, Al-Ali HK, Behre G, Cross M, Niederwieser D: *Prognostic Significance of EVI1 expression in Acute Myeloid Leukemia Patients with intermediate or adverse Cytogenetic Risk undergoing Hematopoietic Cell Transplantation with Reduced-intensity Conditioning,* Presented at the annual meeting 2013 of The American Society of Hematology (ASH) (Abstract #P3383)

- **Jentzsch M**, Lange T, Bill M, Krahl R, Franke G-N, Schakols K, Cross M, Al-Ali HK, Vucinic V, Niederwieser D, Schwind S: *Low BAALC expression associates with better outcome in Acute Myeloid Leukemia Patients undergoing allogeneic hematopoietic Cell Transplantation after Reduced Intensity Conditioning.* Presented at the 12th Leipzig Research Festival for Life Sciences 2013 (Poster #97)

- Weidner H, Bill M, Wildenberger K, **Jentzsch M**, Kloss L, Schmalbrock L, Cross M, Fricke S, Behre G, Niederwieser D, Schwind S: The poor outcome prognosticator ERG is regulated by *miR-9* in Acute Myeloid Leukemia (AML). Presented at the 12th Leipzig Research Festival for Life Sciences 2013 (Poster #91)

- Bill M, Weidner H, Wildenberger K, **Jentzsch M**, Schmalbrock L, Kloss L, Cross M, Fricke S, Behre G, Niederwieser D, Schwind S: *The Transcription Factor ERG (ETS-related gene)regulates the DNA-Methytransferases DNMT3A and DNMT3B and is a novel pharmacological targetin Acute Myeloid Leukemia (AML) patients (pts).* Presented at the 12th Leipzig Research Festival for Life Sciences 2013 (Poster #331)

- Schwind S, **Jentzsch M**, Lange T, Pönisch W, Heyn S, Vucinic V, Franke G-N, Krahl R, Jäkel N, Al-Ali HK, Cross M, Behre G, Marcucci G, Bloomfield CD, D. Niederwieser: *High pre-*

treatment miR-181a-1 and miR-181a-2 expression in intermediate risk acute myeloid leukemia (AML) patients (pts) associates with lower relapse rate after reduced-intensity conditioning (RIC) allogeneic hematopoietic cell transplantation (HCT). Presented at the 12th Leipzig Research Festival for Life Sciences 2013 (Poster #104)

- **Jentzsch M**, Lange T, Bill M, Krahl R, Franke G-N, Schakols K, Cross M, Behre G, Vucinic V, Niederwieser D, Schwind S: Patients with acute myeloid leukemia undergoing reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation: The prognostic impact of aberrant *BAALC*, *ERG* and *MN1* expression levels. Presented at the 120th Annual Meeting 2014 of the Deutsche Gesellschaft für Innere Medizin (DGIM) (#P038)

- Weidner H, Bill M, Wildenberger K, **Jentzsch M**, Kloss L, Schmalbrock L, Cross M, Fricke S, Behre G, Schmidt E, Niederwieser D, Schwind S: The adverse predictor *ERG* is down-regulated by *miR-9* in Acute Myeloid Leukemia. Presented at the 120th Annual Meeting 2014 of the Deutsche Gesellschaft für Innere Medizin (DGIM) (#P033)

- Bill M, **Jentzsch M**, Lange T, Schmalbrock L, Kloss L, Krahl R, Franke G-N, Fricke S, Vucinic V, Pönisch W, Al-Ali HK, Cross M, Behre G, Niederwieser D, Schwind S: *Die Expression des Transkriptionsfaktors EVI1 ist mit einem kürzeren Überleben von Patienten mit akuter myeloischer Leukämie nach allogenerStammzelltransplantation nach Konditionierung mit reduzierter Intensität assoziiert.* Presented at the 120th Annual Meeting 2014 of the Deutsche Gesellschaft für Innere Medizin (DGIM) (#P040)

- Pfrepper C, Klink A, Behre G, Schenk T, Franke G-N, **Jentzsch M**, Schwind S, Al-Ali HK, Hochhaus A, Niederwieser D, Sayer H**:** *Lines of pre-treatment and blast count prior to HCT is predictive for survival in refractory AML patients treated with FLAMSA-RIC.* Presented at The annual meeting 2014 of the European Society of Blood and Bone Marrow Transplantation (EBMT) (Abstract #P020)

- **Jentzsch M**, Bill M, Niederwieser D and Schwind S: *miR-181a Expression in Acute Myeloid Leukaemia.* Treatment strategies Hematology, 2014 Volume 4, Issue 2 (49-55)

- **Jentzsch M**, Bill M, Leiblein S, Weidner H, Wildenberger K, Cross M, Pleß M, Bergmann U, Nehring-Vucinic C, Jäkel N, Krahl R, Pönisch W, Franke G-N, Vucinic V, Behre G, Niederwieser D and Schwind S: *High Burden of CD34+/CD38-cells predicts worse Outcome in Acute Myeloid Leukemia (AML) Patients after Allogeneic Stem Cell Transplantation (HCT) with Reduced-Intensity Conditioning (RIC),* Presented at the Annual Meeting 2014 of The European Hematology Association (EHA), Haematologica 2014;99(s1) 166-167 (Abstract #P502)

- Bill M, Schmalbrock L, **Jentzsch** M, Schubert K, Wildenberger K, Weidner H, Pönisch W, Vucinic V, Franke G-N, Cross M, Behre G, Niederwieser D and Schwind S: *Prognostic Significance of IDH Mutations in Acute Myeloid Leukemia Patients undergoing Hematopoietic Stem Cell Transplantation after Reduced Intensity Conditioning.* Presented at the Annual Meeting 2014 of The European Hematology Association (EHA), Haematologica 2014;99(s1) 167 (Abstract #P503)

- **Jentzsch M**, Bill M, Leiblein S, Weidner H, Schmalbrock L, Wildenberger K, Cross M, Pleß M, Bergmann U, Nehring-Vucinic C, Jäkel N, Krahl R, Pönisch W, Franke G-N, Vucinic V, Behre G, Niederwieser D and Schwind S: *Ein hoher CD34+/CD38- Anteil bei Diagnose einer Akuten Myeloischen Leukämie assoziiert mit einer schlechten Prognose nach allogener Stammzelltransplantation mit reduzierter Konditionierung.* Presented at The Annual Meeting 2014 ot the Deutsche Gesellschaft für Hämatologie und Onkologie (DGHO) (Abstract #V150)

- Schmalbrock L, Bill M, **Jentzsch M**, Schubert K, Wildenberger K, Weidner H, Pönisch W, Vucinic V, Franke G-N, Cross M, Behre G, Niederwieser D, Schwind S: *Isocitrat Dehydrogenase (IDH) Mutationen sind bei Patienten mit akuter myeloischer Leukämie (AML) und ungünstigem Karyotyp nach allogener Stammzelltransplantation mit reduzierter Konditionierung mit einer besseren Prognose assoziiert.* Presented at The Annual Meeting 2014 ot the Deutsche Gesellschaft für Hämatologie und Onkologie (DGHO) (Abstract #V110)

- Tumewu T, Franke G-N, Schwind S, Jäkel N, Heyn S, Pönisch W, Al-Ali HK, Leiblein S, Krahl R, **Jentzsch M**, Behre G, Niederwieser D, Vucinic V: *Allogeneic Stem Cell Transplantation with Reduced-Intensity Conditioning is a Feasible Therapeutic Option in Older or Comorbide Patients wirh Acute Lymphoblastic Leukemia*. Presented at The Annual Meeting 2014 ot the Deutsche Gesellschaft für Hämatologie und Onkologie (DGHO) (Abstract #P174)

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